

France in trouble at Auckland

French security agents, innocent or otherwise of the sinking of Rainbow Warrior, have done more for Greenpeace than any other publicity stunt has done. Not only France will suffer.

THE only certain outcome of the assorted escapades of French secret agents in New Zealand earlier this year is the reverse of what was intended: Greenpeace, the organization whose chartered trawler was sunk in Auckland harbour on 10 July, has been made much better known than could ever have been accomplished by its own publicity stunts. It is a fair guess that when the time next comes for the organization to hire a vessel to attend on a seal-culling in Canada (or elsewhere), the dumping of some material in the sea or the explosion of nuclear weapons beneath Pacific atolls, there will be even less difficulty than there seems to have been in the past in raising the charter fee. And these unintended side-effects apart, the sinking of *Rainbow Warrior* and the consequent death of a photographer with the ship has disreputably made respectable an organization whose claims on serious public attention have hitherto been only marginal. Not only the government of France will rue that development.

Greenpeace is a kind of maritime version of most environmentalist action groups. Just as terrestrial environmentalists will lie down in front of bulldozers bent on building a new airport runway, so Greenpeace will send ships to make a nuisance of themselves at places in the sea where unwelcome operations are intended. Usually, given the traditional freedom of the seas, Greenpeace ships stay within the letter of the law. In some connections, these activities may even be valuable; it will be interesting to see what will come out of the planned attempt to monitor Iceland's decision to capture up to 500 whales in the North Atlantic during the coming year in the interests of what is said to be "research". But the case for sending ships to the French nuclear testing site in the Pacific can consist only of the wish, by publicity attendant on trouble, to make better known what most people know already, that France is a nuclear power, like Britain, China, the Soviet Union and the United States. But nothing in the planned expedition to Mururoa Atoll will do much to give wider currency to the reprehensible feature of France's nuclear policy, its apparently unchanging decision to stay outside the framework of the Non-Proliferation Treaty (and no maritime expedition could hope to do the same in respect of China, a non-member that conducts nuclear tests beneath dry land).

To the extent that what is called "action" of the kind intended at Mururoa functions only by the publicity it engenders, and because the publicity invariably takes the familiar line of the David versus Goliath story (brave voluntary organization chased off by government might), the result is usually to muddy public discussion of important issues. The plain truth is that what France is doing at Mururoa is just as consistent with international law as the testing programmes pursued openly by the four other nuclear powers. Moreover, there is not the slightest possibility that these programmes, symptoms as they are of military reliance on nuclear power, will go away until there is some

general agreement among the major powers on the role of nuclear power in military security. That is one important issue running through the series of negotiations now under way at Geneva, the review conference of the Non-Proliferation Treaty among them. Before the sinking of *Rainbow Warrior*, the seriousness of Greenpeace's motives was rightly questioned. Now, the general wave of outrage that such a thing should have happened will lend spurious credence to a dubious business. Can that be what France intended?

None of this implies that French agents were actually responsible for planting the two limpet mines that sank *Rainbow Warrior*. That question is unlikely to be settled even at the trial of the two agents arrested in Auckland on 17 July and due to reappear in the New Zealand courts on 4 November. But the issue has certainly not been laid to rest by last week's report by M. Bernard Tricot to the President of France, M. François Mitterrand, the most striking characteristic of which is its lack of judicial authority. M. Tricot's report makes plain the reasons why at least six agents were sent to New Zealand, as given by the officials to whom he spoke. The report goes on to acknowledge that the circumstances of this substantial French secret presence in New Zealand is open to sinister interpretation but then proceeds to give the opinion that these unwelcome interpretations are improbable. M. Tricot says that while the orders given in writing to the agents may have been ambiguous (depending on whether the French verb *anticiper* is read as transitive or intransitive), he thinks it unlikely that they will have been understood as instructions to sink the ship, and that the chances of their having been stiffened verbally are small. Similarly, he adduces circumstantial evidence for thinking that the two separate groups of agents could have colluded to sink *Rainbow Warrior*, but there is nothing in his report to suggest that the agents to whom he had access were cross-questioned on the matter. The report is unconvincing because of the internal evidence that M. Tricot's enquiries stopped short at the point at which they might have become embarrassing. No report at all would have been wiser.

Curiously, neither M. Tricot nor the spokesmen who have since been giving official interpretations of the *Rainbow Warrior* affair have dealt with the most disturbing of its features, the assumption (by France, on this occasion) that it is entirely permissible for governments to send their secret agents to take up clandestine residence in ostensibly friendly countries. It is true, of course, that for many years New Zealand has been complaining about French activities at Mururoa; now the ASEAN group of south-east Pacific governments led by Australia and New Zealand is trying to create a non-nuclear zone in the region, at least in part because they hope to shame France into compliance. But is that a sufficient reason why France should kit out agents with fake passports to spend what must have been a fake vacation in New Zealand? Without apparently telling Mr David Lange or his officials what was afoot? There can hardly, in retrospect, have been a more sure recipe for deepening hostility between France and the governments of the south-east Pacific. And there can hardly be a better reason why some general code of conduct for secret agents should be part of an agreement between governments designed to make the world a safer and more seemly place (see *Nature* 29 August, p. 753). □

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, they should in future send **four copies of all manuscripts** offered for publication either (as at present) to London or to Washington.



Advancing science?

Australia, Britain and New Zealand have more in common than is apparent.

BRITAIN and Australia are at opposite ends of the Earth, but that does not entirely explain why there should apparently have been such a contrast between the mood of the autumnal meeting of the British Association for the Advancement of Science (BA) at Glasgow and the meeting in Melbourne last week of its antipodean equivalent, the Australian and New Zealand Association for the Advancement of Science (ANZAAS). Nor is it a sufficient explanation that the two scientific communities have been differently dealt with by the governments that support them; the British scientific enterprise has been under pressure for years, but the Australian government was last year just as beastly to publicly-supported science, while that of New Zealand nurses foolish ambitions to make much of its public science earn a living from the private sector. Indeed, in all three countries, there is now a general clamour from outside the research community that creative people should be more diligent in the future than has been their custom to see that intellectual innovations become saleable products as quickly as possible. (Mostly, the three scientific communities concerned seem willing to respond.) The true explanation for the gloom at Glasgow is that given by Sir Hans Kornberg, the retiring president of the BA, in his presidential address (see p. 4); morale in Britain is in poor shape, and there is nothing in sight to suggest recovery is round the corner; in Australia and New Zealand, by contrast, hope of improvement persists, fortified by this year's Australian budget with its sensible if modest increases for some kinds of support.

The British problem is too deep-seated to be as easily resolved. And money is not everything. Sir Hans Kornberg made that clear last week, by acknowledging that even an improbable decision by the British government that there should be an increase in the support for science sufficient to restore the sense of largesse prevalent in the 1970s would be only a mixed blessing; laboratories might then be equipped well, and sound research grant applications would mostly be supported, but "our present failure to profit from our past discoveries" would probably be perpetuated. Logic leads inexorably from these assumptions to the conclusion that research needs a substantial sponsor other than the public purse, and that far-sighted industry is the most obvious and desirable source of support.

So far, so good. The virtues of plurality are so obvious that nobody will quarrel with the notion that sources of support other than the government are welcome, while there is a wealth of evidence that industrial companies can make grants to academic researchers without seeking to constrain their academic independence. (In Britain, it tends to be forgotten that as recently as the early 1950s, the chief source of postdoctoral fellowship support was an industrial company, ICI Ltd.) But there are two general worries about even enlightened industrial support, without too many strings attached, for basic research.

First, and of necessity, it is impermanent, determined as much by the passing prosperity of industrial companies as by the need. (Contracts between industrial companies and academics for applied research are in a different category, in principle at least.) Second, again almost by definition, the industrial companies willing to back basic research in universities are a restricted group, those willing to recognize that their own well-being ultimately depends on the health of the scientific enterprise and also prepared to make long-term investments in the process of discovery while recognizing that such practical benefits as may emerge are just as likely to flow to their competitors as to themselves. In a world in which the basis of company law enjoins commercial enterprises to acknowledge their obligations to their stockholders rather than to unrelated groups of people such as academic researchers, it is unreasonable to expect that industrial support for basic research will ever be more than a small percentage of what governments such as even the British now spend. Industrial research contracts for specific purposes are different.

What this implies, in Britain as well as the ANZAAS countries, is that governments will have to continue to provide the chief support for basic research, whose virtues and excitements Sir Hans Kornberg properly extolled last week. At least in Australia, the research community seems to have learned quickly that it can help to stiffen the government's resolve to do the decent (and prudent) thing by making sure that people at large are aware of the intellectual excitement of their work, and of its potential value to the country as a whole. Part of the British trouble is that people are often too despondent to tell a convincing tale, but there is also something in the complaint that the leadership of the scientific community has fallen into the hands of people who are no longer active at the bench, and who therefore have only second-hand tales to tell. (Kornberg is an exception, so too is the present president of the Royal Society.) The result is that arguments about government policy are too much concerned with form, and too little with substance, intensifying able people's sense of neglect.

In all three countries (Australia, Britain and New Zealand), the objective should not be over-modest. In all, governments should be persuaded to recognize what they ask industry to acknowledge — the importance of present research as a guarantor of future prosperity, whose nature is necessarily indeterminate. The United States and France, and in very different circumstances, India, have taken this point, with the result that spending is linked not with present wealth but with future expectations. But in each country, there are special problems. Australia resembles Britain in being seriously hampered by the weakness of its entrepreneurs, New Zealand is embarked on an eccentric experiment (making users and putative users pay almost all the cost of research) while Britain, which has woken up to the need to turn an honest penny, cannot get from where it is to where it would like to be for lack of government help over the intermediate hump. Making a social case is not always special pleading. Failing to make a special case whose strategic importance is as great as that for research is a kind of self-inflicted damage. □

Nuclear white elephants

Too much nuclear plant seems to be being built to give construction companies work to do.

IF the Nordrhein-Westfalen government eventually decides not to sanction the loading of fuel into the Kalkar fast reactor (see p.8), it will at least have the comfort of knowing that this expensive machine is not the only one of its kind to have been built but never used. Austria owns a nuclear reactor that has been ready to produce electricity for the best part of five years but is unlikely ever to be used. Among similar unused machines in the United States, that at Shoreham on Long Island is perhaps the best known, chiefly for the continuing public argument about the feasibility of evacuating nearly a million people if there should be an accident, but there is a whole clutch of unfinished nuclear reactors in the north-west that are unlikely ever to be completed. At just over DM 7,000 million, Kalkar is relatively cheap among its kind, as befits an intended demonstration reactor.

Even so, the SPD government in Cologne should think twice before throwing Kalkar on the rubbish heap. The project has been so much delayed already that its value as a means of proving a novel nuclear technology can only be small at this stage; the largely French *Superphénix* project, for example, has probably more to offer those who will next century go back to the fast reactor technology. One danger in what the regional government seems to have in mind is that there is no sound technical reason why it should not wring a little understanding from the reactor. Worse still, the calculation is in at least some politicians' minds that putting a stop to Kalkar will win friends among supporters of the West German Green party, and thus advantages at the federal elections due in 1987. Not merely is the calculation cynical, but it could backfire. Kalkar is not the only project in Nordrhein-Westfalen unpopular with the Greens. □

US textbooks

Californian setback for evolutionists

Washington

THE California State Board of Education is about to take what could be a decisive stand against bowdlerized US biology textbooks that give only a passing mention to evolution. The board's curriculum commission has decided that none of the life science books submitted for approval for the \$25 million Californian school textbook market are worthy of adoption, and has told their publishers to go away and try harder.

California is only one of several states that have statewide approval of junior-school textbooks, but although it is the largest market, publishers have tended to slant their books for the more conservative states, especially Texas, which until recently forbade any mention of evolution in biology books used in state schools. California, on the other hand, has adopted a model curriculum that includes dating of fossils and the principle of natural selection for the 11–14 age group. Although all the books submitted for approval in California for this age range mention evolution, in many it is confined to a few pages, according to reviewers.

The most plausible explanation for the reluctance to discuss evolution is that publishers hope to escape the attentions of the creationist lobby, which is enjoying a nationwide revival, buoyed up by a growing tide of religious fundamentalism. Publishers naturally wish to avoid having to produce different editions for different states. But, in the words of one Californian legislative analyst, the commission is now "sending a signal that they might be able to sell those kinds of books in Texas or Alabama, but California will not accept less" than the model curriculum dictates.

The efforts made in the books to avoid anything that could be taken as a clear statement about evolution have given rise to some memorable nonsenses, some of which have been collated by William Benetta, an independent editor who gave evidence to the commission. Characteristically, the word "evolution" is not used except when prefaced by "theory of"; thus Charles Merrill's *Focus on Life Sciences*, which omits evolution from its glossary, declares that "the theory of evolution can be explained by mutations and natural selection", while Scott Foresman and Company's *Life Science* avers that "scientists call such changes in groups of organisms over time the theory of evolution". Addison-Wesley's *Life Science* is even more obscure; a passage that attempts to link mutation and natural selection without mentioning the forbidden word at all concludes with the tantalizing assertion

that "under some conditions mutation may cause a change in an entire population of living things". The Addison-Wesley book was not recommended for adoption by the State Board; others that were recommended with the proviso that their coverage of evolution be improved were published by, in addition to Scott Foresman and Merrill, Prentice-Hall, Macmillan, D.C. Heath and Holt, Rinehart and Winston. Some publishers were also told to improve coverage of human reproduction and environmental and ethical issues.

Books for younger children were also criticized by the commission. Many of these discussed reproduction in animals but neglected to depict or discuss the relevant basic anatomy in humans, so again falling foul of the State Board's model

curriculum. The publishers have the option of preparing "supplemental materials" to make good these omissions.

The State Board meets to consider the curriculum commission's recommendations on 12 September but some members have already let it be known they are impressed by the commission's diligence. If, as expected, the recommendations are accepted, publishers will have until 1 February to produce revisions of their texts. But, in doing so, they will have to avoid going too far in the opposite direction, since a Californian state law forbids dogmatism in teaching of theories about human origins. Some education board specialists think that creationists, having lost the battle to have "creation science" taught as such, will try to sue the state under the antidogmatism law if evolution is presented credibly. Publishers have every incentive to get this balancing act right, however. Californian school textbooks are approved on a multi-year cycle that rotates by subject, and science books approved in the current school year will be the only ones used in state schools for the next six years.

Tim Beardsley

NSF

Change of direction afoot

Washington

THE US National Science Foundation (NSF) has embarked upon what may turn out to be an important shift in the way it funds research. The foundation has started supporting a series of small materials research groups on university campuses, in which typically fewer than 10 investigators will collaborate on a single shared project. The new groups provide an alternative to the existing much larger Materials Research Laboratories, in which several different areas of research are pursued at one time.

Initial funding of the materials research groups is for three years. The institutions supported and the research areas are as follows: Rensselaer Polytechnic Institute — stability of glasses; Polytechnic Institute of New York — ageing in polymer blends; Pennsylvania State University — chemically bonded ceramics; University of Texas, Austin — photochemical process at interfaces; California Institute of Technology — motions of atoms and molecules at interfaces.

Although the amount of money committed to the new scheme so far is small (\$8 million), this will increase rapidly as new groups are set up. Furthermore, other subjects may follow the example set in materials research; the NSF chemistry division, for one, is expecting shortly to increase support for small collaborative groups, and engineering may not be far behind.

At present, NSF spends something close to 40 per cent of its \$107 million materials research budget in the Materials

Research Laboratories, which were originally established in the 1960s by the Department of Defense. Most of the rest goes to individual investigators. But the laboratories, each with a budget of several million dollars, have been criticized for failing to fulfil their intended role of fostering interdisciplinary research. Rustum Roy of Pennsylvania State University, a prominent champion of materials science research, believes that rigid university departmental boundaries continue to be a "major impediment to national needs" and says that Materials Research Laboratories have failed to assimilate many of the most important recent developments in the field, such as advanced polymers, high performance ceramics (where "the British are clearly ahead") and gallium arsenide. Roy dismisses some of the existing Materials Research Laboratories as little more than bureaucratic fictions and characterizes the new materials research groups as a praiseworthy if belated attempt to "seduce universities into accepting interdisciplinary research".

NSF officials do not see things in quite that way. Two of the fourteen existing Materials Research Laboratories (at Ohio State University and Purdue University) are now being phased out, with no immediate plans to replace them, but there is no plan to supplant the big laboratories with the new research groups, according to Lance Haworth of NSF's division of materials science. Rather, he says, the groups will be a halfway house for building up to or down from full laboratory status.

Tim Beardsley

British Association

What price excellence?

Glasgow

FOR the second year running, the blue-and-white insignia of the British Association of Science match the pyjamas of its president. Such complementary colouring, as Sir George Porter, the new president, assured his predecessor last Friday, is intended to encourage dedication to the job. Given the depressed state of basic science in Britain, as described during the British Association (BA) audit on science spending, Sir George should have many opportunities to coordinate his silver pendant with his nightwear.

Sir Hans Kornberg's opening address to the BA's annual meeting as outgoing president set a sombre tone at the University of Strathclyde (Glasgow) last week. One index, he says, of the "horrificing rate of decline" of British research is the inability of the Science and Engineering Research Council (SERC) to fund three-fourths of its finest research grant applications. He was the first of many to defend the role of basic research in improving Britain's industrial and scientific health. Science must be put to use (the primary theme of the meeting), in other words, but at what cost? By making "purely academic research by people motivated solely by the desire to know" the scapegoat for the failure to profit from this research, Britain would moulder as an "exploiter of imported ideas" and an "assembler of imported parts".

A week of rain and mud up to the knees on visits to scientific sites created a proper Shakespearean backdrop to these discussions. The host university, however, is used to such weather and showed many signs of spirit. Mr Hugh Thomson, head of Strathclyde's Research and Development Services, expressed high hopes for the fledgling science park, as well as pointing to the £1.5 million in research contracts recently won from the European Economic Community, an amount far above average for British universities. And where else besides Glasgow is there a Full-Scale Structures Testing Unit, which does not need much money because there are so many derelict buildings in Scotland just waiting to be blown up? In this context could be found discussions on everything from topics of the day, such as cancer, acquired immune deficiency syndrome (AIDS) and acid rain, to the censoring of isolated naked quarks.

Studying quarks, be they naked, down or up, sparked an ongoing debate on the proper role of public opinion in science funding. "Not with my money" is a public response dangerous to basic scientists, whose work often has no obvious immediate value for Britain's well-being. As Professor G.G. Roberts, head of applied physics at Durham, said, research in such fields as quarks "should be restricted" un-

til the United Kingdom "can afford the luxury of financing them".

At the science audit, Sir David Phillips, chairman of the Advisory Council for the Research Councils (ABRC), outlined the history of another kind of planning blight. He pointed to a scientific malaise in industrial countries, caused by the necessary reduction in their percentages of world scientific activity as the Third World develops. The science vote in Britain increased in real terms by a factor of 50 since 1930, but has now levelled off. Only "outmoded attitudes" lead scientists to expect a return to expansion.

There was no paucity of fingers pointing at causes for the decline in British research. Professor C. Hilsum, chief scientist, GEC Research Laboratories, for example, claimed that Britain's financial institutions make competing with the Japanese more difficult. British companies, he says, have an average of one-third the fixed assets of Japanese companies, partly because they expect a much higher return on those assets than do the Japanese. The high ownership of shares in UK businesses by pension funds and investment trusts (58 per cent as opposed to 20 per cent in Japan) encourages "liquidity without commitment, more finance and less business".

Industry is an obvious target. According to Sir Hans Kornberg, industry should recognize the importance of basic research in universities to its own growth and thus provide more support at an early stage. The research divisions of companies are also an important link between basic and applied science. Mr R.A. Street from the Palo Alto Research Center in California described these divisions as an avenue for attracting top people to the company and as a door to the universities. Dr I.A. Shanks from Unilever outlined how, when these doors close, ideas from outside can be strangled by industrial fear of unprotected ideas and new market areas.

The research councils and their methods of funding are a problem very close to home for the scientists they support. These councils were set up after the First World War, according to Sir David, as a means of improving the economic performance of Britain. Since then they have been described as too compartmentalized properly to compare competing sources for funds as well as too outdated to analyse multidisciplinary research such as biosensors. SERC, for instance, is divided into nuclear physics, astronomy and space science and, belatedly, engineering. With the slashing of budgets, nuclear physics has been a dramatic loser.

The audit had an aura of stewing in its own juice. There are too few carrots available for prodding the development of a

national science policy, though all too much evidence of what happens without one. The feeling of getting nowhere, of finding no solution, led one nuclear physicist in the audience to proclaim, "We may be the first to go but we won't be the last".

How, then, should excellence be identified? One method, now much in vogue, that adds statistical backing to peer reviews and interviews is citation analysis. These data can show both trends in fields and excellence of individual researchers. For example, an analysis of the most cited papers across all fields by the Institute for Scientific Information in Philadelphia shows "a distinct absence of physics and chemistry" papers from the United Kingdom but strength in biomedical and biological areas. ABRC has funded several citation analyses from Ben Martin and John Irvine of the Science Policy Research Unit (SPRU) of the University of Sussex.

Given the shrinking UK funding pie, however, excellence cannot be properly supported in all fields. Who and by what means those choices can be made are questions that promise to draw blood. "No one institution could cope", says Sir David, but at the same time "bottom-up" procedures suffer from narrow specialization. He set forth another option: discussion among all the fields involved, including all interested parties, which would result in a "consensus that needs no other implementation". Dr Hilsum also pointed to the Japanese forecasts that create national goals and thus national achievement.

Interest in this avenue for national policy making has been stimulated by Martin and Irvine's book *Foresight in Science*, which resulted from a study funded by ABRC on Japanese policy formation. It describes how the Japanese Science and Technology Committee gathers responses from active researchers, over half in industry, on what is likely to be achieved in these fields. National goals result, which, because of the previous participation, are more easily accepted. This option was not taken seriously in the BA audit discussions, but it is likely that ABRC will try out a method similar to that described by Martin and Irvine.

The large number of people at the physics sessions on the beginning and future of the Universe were a telling comment on criticism of basic research, even if the precise role of the down quark is not essential for UK industrial growth. Maybe, on the other hand, they were there because the axioms were different. In the new big bang theory as described by the quantum physicist Professor P.C.W. Davies, activities do not have to have well-defined causes, the big bang is an "inflation" (used in a positive sense) of a whimper into empty space and energy is created from nothing. An even pleasanter thought was Dr Davies' support of a statement from Dr Alan Guth of MIT: "the Universe is a free lunch".

Elizabeth Collins

ANZAAS

Selling public views of science

Melbourne

THE annual congress of the Australian and New Zealand Association for the Advancement of Science (ANZAAS) has splendidly reformed itself. The usual format was turned on its head at this year's meeting, held at Melbourne's Monash University from 26 to 30 August. The meeting, one week after the Commonwealth government of Prime Minister Bob Hawke had announced its annual budget, provided a timely opportunity for assessing the mood and direction of Australian science and for scientists to make a wider pitch for public support.

At this ANZAAS congress, the 35-odd specialized sections that have dominated the proceedings of ANZAAS for most of its 97-year history as the one organization in Australia and New Zealand attempting to encompass most areas of academic expertise, had disappeared. In their stead, the organizers, led by the energetic congress director, Professor John Swan (chemistry, Monash), built the programme around 129 interdisciplinary symposia focusing on current social issues.

Among the more popular sessions were space science and technology, star wars and new astronomy (in the "hard" category), acquired immune deficiency syndrome (AIDS), sexually transmitted diseases in general and occupational health and safety, schizophrenia and the fluoridation of water supplies and the shape of things to come.

The congress was directed firmly towards a wide general audience, while retaining authority with reputable speakers. Recent congresses have tried, with little success and limited academic respect, to act as meetings for announcing original work. The dominance by social scientists at previous meetings (74 per cent of the speakers at the 1984 congress) was corrected; straight scientists this year comprised 51 per cent of the 700 speakers.

To this observer, seasoned by two decades of ANZAAS congresses, the new formula succeeded splendidly. The outreach to the public was a massive improvement. And the scientific community still turned up.

This congress was expertly marketed and attracted large numbers to the Monash campus, despite its remoteness in the flat suburbia of Melbourne. Instead of an all-up registration fee, there was a computerized ticket system handled by a commercial ticket agency. Anyone wishing to attend a half-day session paid \$A8 for entry (students and pensioners \$A5).

The risk was that the organizers had no idea beforehand whether academics and the public would respond, especially given the difficulty of getting to Monash, 20 km from the city centre. In the event, the 15-17 concurrent sessions were mostly

well attended, with 100-plus audiences, a pleasing increase on the often embarrassingly small sessions of the last traditional congress in Canberra last year. The total number of tickets sold — some 7,000 — was estimated to show that at least 3,500 different people attended the formal sessions, a useful improvement on the number of registrations of under 3,000 at congresses over the past decade.

This congress was billed as a "festival of science" and lived up to its ambitious title. A range of events extended well beyond the limitations of the campus-bound symposia. There was, for example, a week-long special programme for senior high-school students, called "Youth ANZAAS", which drew nearly a thousand young people to a programme launched by the New Zealand folk hero, Sir Edmund Hillary, the president of this year's congress.

The reach of science into the community was extended with a "Community Science and Technology Program". The offer of free tickets to visit some 80 science-based institutions throughout Melbourne drew 14,000 takers. There were displays of "everyday science" in the main city square in the week before the congress, and students could follow a "mathematical trail" around the city. One of the most popular events was the "hands-on" science circus, the embryo nucleus of the National Science Centre based at Canberra with a travelling component. All told, ANZAAS activities reached at least 22,000 people directly, nearly eight times as many as at previous meetings. Indirectly, millions of Australians were reached through the extensive media coverage before and during the meeting.

In the present political climate, the significance of the achievement is that it epitomizes a new assertiveness among some of the more politically aware scientists. The 1985 budget dealt relatively kindly with science; last year's cuts, which so aroused the research community, were at least not repeated. This may be partly the result of the effort by scientific organizations to lift their public game. Thus the Australian Academy of Science has improved the public profile of science through the establishment of its national committee for promoting science and technology. And the largest research institution in Australia, the Commonwealth Scientific and Industrial Organization (CSIRO), which had suffered public opprobrium and financial cuts, directly lobbied key ministers other than their relatively lowly ranked Minister of Science, Mr Barry Jones, after the loss of the technology wing of his portfolio. Mr Jones himself shows a new enthusiasm for promoting fundamental research. At ANZAAS last week, he said that the post-

1984 budget period was "the political Death Valley for Australian science. Scientists were acting like nocturnal animals, being startled by loud noises and retreating under threat, so you cannot see them." After the 1984 budget, Mr Jones had jolted scientists by labelling them as "the wimpiest collection of lobbyists". Now, both he and his opposite number in the Liberal-National Party opposition, Mr Michael MacKellar, agree that the increased visibility affects budget fortunes. But there is a long way to go.

Through the academy's promotion committee, and again at ANZAAS, plans have been presented for a science and technology information service (for the media, politicians and science educators), a science and government relations bureau, a regular series of polls for gauging and responding to the public opinion of science and scientists, and a book covering Australian science and technology in terms accessible to decision-makers and the public. Some of the running may be made by the Association of Science Communicators of Australia, formed at ANZAAS. Next year, when there will be no ANZAAS congress (the next congress will be in January 1987 at Massey University, New Zealand), the new association plans to hold a national conference instead.

While this ANZAAS congress has been a success, the parent organization struggles along with a low membership and an inadequate budget. Its largest problem is that of keeping alive its monthly journal *Search*. Peter Pockley

FDA division to close

Washington

THE Food and Drug Administration (FDA)'s Drug Chemistry division in downtown Washington, DC, is once again scheduled for closure, apparently as a consequence of pressure within FDA for more resources to be devoted to reviewing New Drug Applications.

The laboratory had one close shave two years ago, when a threat of closure was averted after unfavourable publicity, but this time the deed has been done quietly. Thirty chemists are employed in the division.

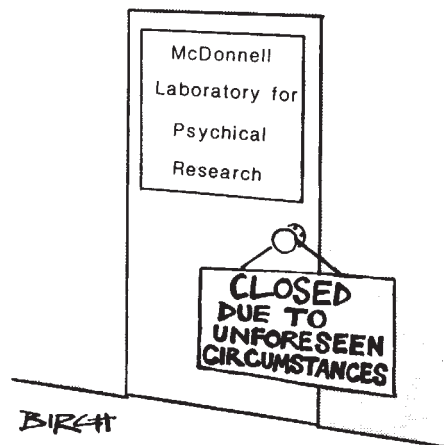
Most of the functions of the laboratory, which include developing drug reference standards and new applications for instrumentation, will be transferred to the FDA laboratory in St Louis, Missouri. This will release 20 jobs in Washington to be turned over to reviewing drug applications. But some of the work seems likely to be lost. The division's general methods branch, which has developed new methods of separating stereoisomers, is unlikely to be recreated elsewhere. Two scientists in this division, Irving Wainer and Tom Doyle, received FDA awards for scientific excellence last year. Tim Beardsley

Parapsychology

MacLab, St Louis, to shut

Washington

AFTER six controversial years, the McDonnell Laboratory for Psychical Research at Washington University, St Louis, finally closed at the end of August. The equivocal claims of evidence for extra-sensory perception (ESP) that have emerged from the laboratory have apparently not been sufficient to persuade heirs of the late James McDonnell, a devotee of the paranormal, to continue giving financial support to parapsychology.



The laboratory, known as MacLab, never fully recovered from the shock of James Randi's Project Alpha in 1983. Randi, a professional magician and foe of sloppy parapsychological research, sent two protégés into MacLab posing as gifted young psychics; the two were investigated at the laboratory for three years before Randi announced the deception at a press conference.

Rancour persists between Randi and the director of MacLab, Peter Phillips. Phillips admits that he was deceived, but says the laboratory never published a claim that it had found psychic abilities, and that he eventually devised experimental protocols that defeated the self-proclaimed psychics. He says Randi used Project Alpha for self-advertisement. Randi, for his part, says Phillips had no idea what was going on until he was persuaded to use protocols supplied by Randi himself; he also says Phillips prepared a printed report claiming psychic effects that was withdrawn once Randi had shown how easy it would be for a magician to cheat.

Since Project Alpha, the research at MacLab has been carried out by a research student Michael Thalbourne. Thalbourne says he has not found any unequivocal, repeatable demonstration of ESP but that evidence for it has surfaced several times in unexpected ways. But according to Randi, Thalbourne's research is still anything but meticulous and Thalbourne has a tendency to re-examine data after the fact for significant effects. He accuses the experimenters of having a

prior conviction that ESP effects are there to be discovered which leads them into error.

The McDonnell Foundation's original bequest (of \$500,000) was intended to run MacLab for only five years, later extended for a further year. Randi says he would

have liked the laboratory to continue, but to be "run by good scientists".

Thalbourne will now continue his experiments out of his own pocket, while Phillips is off to China to investigate "extraordinary" reports about physics there. But Randi claims to know already about the Chinese reports and says they are explained by a very simple sleight-of-hand deception, which he has not yet passed on to Phillips.

Tim Beardsley

Technostress

Another Japanese discovery

Tokyo

"TECHNOSTRESS" is a medical condition that will not be found in the textbooks, but which the Japanese are taking seriously — indeed they bear responsibility for adding the word to the "English" language. Popular magazines are full of articles on what to do about it and a patent baldness remedy warns that at the very least the "technostress era" will make all your hair fall out. And the Ministry of Health and Welfare has now announced that a full scale study is to be launched to find ways to alleviate it.

Technostress is the stress and concomitant psychosomatic disorder induced by the introduction of high technology. Usually high technology means office automation: it includes the stress produced by working at rhythms dictated by machine, the strain of spending a day looking at a video monitor, and the loss of self-esteem of those who find themselves unable to master new equipment or those who, as one Japanese put it, were "replaced by a thing no bigger than a box of matches".

The study has been launched after several reports of sharp increases in the number of patients who work with or around computers admitted to hospital with psychosomatic disorders. A recent survey carried out at Nihon University also showed that close to half of all visits to general medical practitioners resulted from stress-related disorders.

The first aim of the study will be to try to unravel how stress affects the incidence of disease at different stages of the life cycle. There is already considerable evidence that high blood pressure and ulcers are related to stress but links will also be looked for in disorders such as heart attacks and cancer. And perhaps more important, a ministry official said, is that the study will put an end to thinking of many disorders as being either physical and requiring the treatment by a doctor, or mental and requiring treatment by a psychiatrist. It is hoped that environments that minimize stress can be designed and perhaps counselling services provided that will give advice on how to deal with stress-related disorders.

Alun Anderson

Indian astronomers to help Soviets

New Delhi

INDIA, which has been invited to join the International Halley Watch, has also signed a protocol with the Soviet Union for a joint study of Halley's comet during its appearance in 1985–86. Data from Indian observatories will help Soviet astronomers to make mid-course corrections of the Vega spaceprobes now on their way to meet the comet in mid-March 1986. The protocol also covers collaborative observations from both Indian and Soviet ground stations for polarization measurements, speckle interferometry, and infrared and radio observations at decametre wavelength.

The national programme is being coordinated by the Indian Institute of Astrophysics in Bangalore which is operating the 40-inch reflector telescope at Kavalur near the east coast in Tamilnadu state. Apart from Kavalur, the programme centres around the Nizamiah Observatory in Hyderabad, which has a 48-inch reflector, and the 40-inch telescope in the solar

observatory at Nainital in the western Himalayas. According to Professor K.R. Sivaraman of the Indian Institute of Astrophysics, who is the Indian representative in the International Halley Watch, the unique locations of these observatories was a reason for India being invited to join the international programme. The longitudinal gap between Chinese and Soviet observatories is expected to be bridged by the Indian stations.

According to Professor Sivaraman, the planned observations include astrometry, the comet's spectroscopy and infrared studies. Scintillation studies of radio sources occulted by the comet's tail are planned using the radio telescope at Ooty on the Nilgiri Hills in south India. Another radio telescope at Gouribidanur near Mysore will be used to observe the comet at decametre wavelength. The Tata Institute of Fundamental Research will fly balloons carrying telescopes up to 30 kilometres height for infrared observations.

K. S. Jayaraman

Halley's comet

US commercialism runs riot

ALTHOUGH few amateur astronomers—or professional ones, for that matter—have yet espied Edmond Halley's comet in its 1986 apparition, there can be few members of the public in the United States who have not yet woken up to the fact that something unusual is soon to appear in the heavens. Not since Apollo 11 landed on the Moon has the public consciousness been so deluged with astronomical facts and figures, and the phenomenon is rapidly becoming an excuse for a nationwide party.

Parties need party suppliers, and suppliers have sprung up for every conceivable comet-related purpose. The Halley's comet T-shirts were perhaps inevitable (at least a dozen different varieties are available), although the manufacturers have not taken the apparently obvious opportunity to save money by sticking for once to monochrome; full 4-colour images of the comet, with some artistic licence, are standard. And the dedicated engravers of unique commemorative medals have of course been hard at work. More surprising, though, have been the comet cola and the comet pills, the latter guaranteed to ward off the harmful effects of comet vapours. The Grand Rapids Public Museum in Michigan, supplier of comet pills, point out that their efficacy has been proved beyond question: there were no casualties among those who took the same pills during comet Halley's 1910 appearance.

The comet has not only given rise to new product lines: whole new companies have been created to take advantage of the marketing opportunities. Bumper stickers, special comet stamps and posters ranging from educational to psychedelic are all selling well. Among the most ambitious of the new comet corporations is Halley's Comet Interplanetary Corporation, which advertises offices in New York, California and the Milky Way, Quadrant 7. The corporation offers its interplanetary market a complete comet party kit, which includes the game "pin the tail to the comet". And, in the country that invented movies, it is perhaps no surprise that Tobe Hooper's "Lifeorce" should be based on the idea that the comet is peopled with strange and rather unpleasant aliens.

For the more serious minded, there will be plenty of opportunity for self-improvement. Hardly a natural history museum in the country is not making some effort to satisfy the insatiable demand for information about Mr Halley's comet. Lectures are standard for Americans, ever keen to educate themselves, and every qualified lecturer has a full tour booked. Planetariums have special shows, usually daily. And book publishers have had a bonanza.

Telescope manufacturers have come into their own. Sales of serious telescopes for amateurs have rocketed (there is likely to be a glut of second-hand telescopes on the market next year). In addition, a number of manufacturers have produced simple refractors aimed specially at would-be Halley watchers, such as the \$200 Halley-scope manufactured by Halley Optical Corporation. At least one telescope advertised as especially suitable for viewing the comet has attained the ultimate accolade in marketing respectability by being included in mass-mailing from the American Express Company.

Although the size of the comet will not be a problem, its brightness will be. Owners of personal computers, however, will be able to choose from a wide selection of programmes that will enable them to track the comet. And for those who do not own computers, there are various other stick-

and-string devices that will compute the comet's location at any given date and hour.

The comet is going to be much more easily visible from the Southern Hemisphere than the Northern, so its appearance has served as an excuse for long and luxurious cruises for many. Bookings are at record levels. The Planetary Society is offering a cruise to Rio de Janeiro with a special discount for members; the cruise includes a four-man team of "eminent astronomers and scientists" who will be available to give personal advice on viewing the comet. The Royal Viking Line offers an Auckland-to-Sydney cruise with the redoubtable Carl Sagan on board and available for consultation. Or, for those who prefer to stay on dry land, there are motor-coach tours in South America. All in all, the comet is being billed as a once-in-a-lifetime experience, which it will be for most. But one cannot help feeling sorry for the many other comets that will come and go entirely unremarked outside serious astronomy circles. **Tim Beardsley**

Erice

Scheme for world laboratory

A "WORLD LABORATORY" devoted to "research without secrecy" could be a major factor in promoting an international climate of openness and sharing, Professor Antonino Zichichi urged last month. Addressing the Erice International Seminar on Nuclear War, Zichichi outlined his proposals for such a worldwide community of scientists which would conduct joint research into the "dramatic problems" facing the world, including the threat of nuclear war, world hunger and political violence. Results of this research would be made available for the benefit of all.

Preliminary work on the concept began last September, so that Zichichi could report to this year's seminar the establishment of working groups on topics ranging from high-energy physics and asteroid encounters to fuel slurry technology and food production. The working groups are now working out programmes, and as each of these is completed, the necessary laboratory facilities and financial support will be sought internationally. Professor Zichichi does not expect any difficulty in getting this backing—the concept of the world laboratory has grown out of the regular international seminars at the Centre for Scientific Culture in Erice, which enjoy the encouragement and support of many internationally eminent figures, including the President of Italy, the Pope and the Secretary of the United Nations. How much real support the world laboratory will enjoy from governments and regimes not strongly committed to international "openness" is not, however, clear; but Zichichi is enough of a pragmatist to realize that "the world laboratory must begin with the possible, it will begin with the

feasible", working initially in those fields where "collaboration without secrecy" does not run counter to government restrictions.

"Words, talks and round tables", Zichichi told the closing session of the seminar, "are totally useless and serve only as illusion makers". Instead of indulging in such activities, scientists should devote their time to the study of how the world laboratory could become a reality. At next year's seminar, he said, he will report back on the support of governments who have "begun to remove this terrible enemy of humanity: secrecy".

But if the world laboratory is to achieve its purpose, it will have to be supported by major powers both East and West. The Chinese delegates to the Erice conference seemed impressed by the concept, according to one participant, although the main result of their presence at Erice so far has been an invitation to one of last year's speakers to visit China to advise on building nuclear fall-out shelters. The participation of Soviet scientists in the world laboratory is also essential, according to Professor Zichichi, and the initial Soviet reaction last September was positive. This year, however, the Soviet delegation failed to arrive at Erice, an absence that many participants linked with the disappearance last March for the Soviet nuclear winter expert Vladimir Aleksandrov during a visit to Spain. Zichichi stresses, however, that he has so far received no explanation of the Soviet absence, but has every hope that further discussions with them about the proposed world laboratory will take place in the near future. **Vera Rich**

West German fast breeder

Kalkar may fall at last hurdle

Bonn

THE future of the uncompleted fast breeder reactor SNR 300 at Kalkar (Niederrhein) in West Germany is still in doubt. The Social Democratic Party (SPD) is contemplating the consequences of refusing planning permission for the 17th step of the construction programme which would mean that nuclear fuels could not be brought onto the site or loaded into the reactor, so making Kalkar, a "masterwork of this century" (according to SPD in the 1970s), the most expensive waste of investment in Europe.

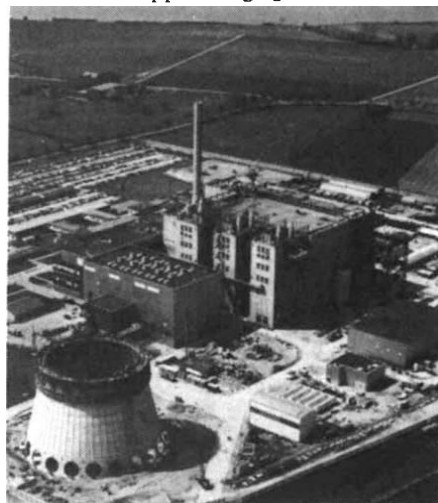
There are many reasons why SPD has now started to express doubts about the project. The permit to store nuclear fuel on the site is seen as irrevocable. According to Friedhelm Farthmann, leader of SPD in the Landtag (state parliament) of Nordrhein-Westfalen, it would be impossible to prevent the reactor being commissioned once it had been loaded, and it is in any case far more expensive to dismantle a reactor site once it is radioactive.

After an overwhelming election victory in Nordrhein-Westfalen, SPD feels powerful enough to act on the decision made at the 1984 federal party meeting in Essen to phase out nuclear technology, and to put an end to the "nightmare of the fast breeder" (SPD in the 1980s). SPD has supported the fast breeder through the previous 16 planning permits. Political pressure from the Green Party and from other sections of the community have certainly played a part in SPD's change of heart. By withdrawing from the fast breeder project at Kalkar, SPD expects to gain significant support from the Greens at the next federal elections in January 1987.

The history of the fast breeder reactor is one of controversy, court cases and enormous increases in estimated costs. In 1970, the former head of the fast breeder project at the nuclear research facility in Karlsruhe and the "father" of Kalkar, Wolf Häfele, estimated that less than DM 500 million would be necessary. As the foundations were being laid in 1973, it was announced that DM 940 million would be required, and that the reactor would be in service in 1978. Even then, the reactor was not expected to produce electricity economically, and the research aspect was brought to the fore. A year later, a spokesman for the fast breeder nuclear energy plant said that the project should be stopped for economic reasons. By January 1976, estimates had risen to more than DM 2,000 million, by August 1977 to DM 2,600 million, by the end of 1981 to DM 5,400 million and the cost is now expected to be about DM 7,000 million.

Ninety per cent of this has to be financed by the taxpayer. The Ministry of Research and Technology, now led by Dr

Heinz Riesenhuber (CDU), had spent DM 3,093 million by the end of 1984; a further DM 418 million is foreseen in this year's budget. The energy industry is responsible for only the remaining 10 per cent; otherwise, according to Farthmann and to August Wilhelm Eitz, head of the construction company Schnellbrüter-Kraftwerksgesellschaft, Kalkar would have been stopped long ago.



The reactor was nearly cancelled once before, in 1978. Then the Economy Minister of Nordrhein-Westfalen, Horst Ludwig Riemer (FDP), refused to issue planning permission for the third building stage; this would have enabled the reactor to produce plutonium and thus to build atomic bombs, which West Germany has declined to do.

At the same time, the federal constitutional court was considering the constitutionality of fast breeder technology. On 8 December 1978, the court decided in favour of the project. After heavy internal dissension within the SPD-FDP coalition, this ruling was followed on 20 December 1978, by Riemer, who was under pressure particularly from the former Chancellor Helmut Schmidt (SPD) and the FDP leader Hans-Dietrich Genscher.

SPD has now found new reasons to delay. Shortly after his election victory earlier this year, Johannes Rau (SPD), the Minister President of Nordrhein-Westfalen and now a likely social democratic candidate as federal chancellor, wrote to Helmut Kohl, the present CDU chancellor, expressing doubts about the present policy. Friedhelm Farthmann was blunter in an interview: he saw "no purpose" in continuing with the fast breeder project, since all of the original reasons put forward to justify it were no longer tenable.

Electricity from the reactor would be more expensive than from conventional sources, including the existing nuclear plants, which produce 30 per cent of East Germany's total electricity. The expected

shortage of uranium supplies has not occurred, and they are now seen to be sufficient for centuries. In addition, since the design of the reactor core was altered, the expected "breeding rate" has fallen to a point where it could never produce fuel for light water reactors.

Klaus Matthiesen (SPD), Minister for the Environment in Nordrhein-Westfalen, was equally blunt. The man who will have to decide whether to grant permission for the latest stage, Reimut Jochimsen, the Economy Minister, was more cautious, although he is reluctant to assent because there is at present no acceptable place or method for disposing of the nuclear waste that will result from loading and running the reactor.

On the other hand, Federal Minister Riesenhuber sees no reason for not completing construction and putting the reactor into service. He feels that cancellation of the Kalkar project would undermine all "sensible support of research". August Wilhelm Eitz thinks similarly: "The object (of the project) is not to produce cheap electricity or extra plutonium but to prove that the system functions as a power plant, as a basis for future power plants of this type."

Farthmann denies however that there is still a useful research element in the project. Another fast breeder in Germany is unthinkable. No similar reactors are planned elsewhere, so that West Germany would not be able to sell its know-how. There would be more interest in the French *Super-Phénix* technology. Wolf Häfele still believes that "the breeder is the only technology that at present allows uranium to be managed with negligible use of resources", denying that the present developments can be extrapolated into a distant future.

Minister Riesenhuber is aware of the seriousness of the opposition. He promised Reimut Jochimsen on 16 August that he would issue a policy declaration about the nuclear disposal problem and about fast breeder technology in general. He insists there will be no problem about issuing permits for nuclear disposal.

The Economy Minister of Nordrhein-Westfalen was more hesitant; apart from the question of disposal, he is not convinced of the political need for the project. Jochimsen will probably make his decision later this month. That decision will probably be the first to throw light on the question whether or not the Federal Republic has sunk DM 7,000 million in a research disaster. If Jochimsen says no, however, there will almost certainly be a protracted lawsuit before the issue is finally settled. The federal government can, if it wishes, force the Nordrhein-Westfalen government to grant a permit. A Land government for its part can object through the courts to such federal instructions. The proceedings would be protracted. And then the fast breeder project could be killed by more delay.

Jürgen Neffe

Hydroengineering

Go-ahead for Danube

THE controversial Gabčíkovo-Nagymaros hydroengineering project on the Danube, halted last February under pressure from Hungarian environmentalists, is to go ahead. But the Hungarian government has pledged itself to "take into consideration" the recommendations of an expert committee, whose members include representatives of the Hungarian Academy of Sciences and the National Council for the Environment and Nature Protection. At the same time, the cooperation agreement between the Czechoslovak and Hungarian academies will be extended to include long-term monitoring of the environmental effects of the dam. Revised completion dates for the hydroengineering projects have been set at 1980 for the 720 MW facility at Gabčíkovo, 1193 for the 160 MW Nagymaros plant, and 1995 for overall completion. Both dams are intended to serve as peak-hour generators, both countries being committed to nuclear power for round-the-clock supplies.

The Czechoslovak side has always been keener on the completion of the project, principally because of the benefits from the flood-control effects; the damage to the water table of northern Hungary, on the other hand, may cost more to remedy (by providing alternative drinking water supplies) than the estimated saving in energy costs. There is also the considerable psychological factor (for the Hungarians) that, as a result of the scheme, the main navigational channel of the Danube, which at present forms the international frontier between Czechoslovakia and Hungary, will be diverted into Czechoslovak territory, while the course of the frontier will be marked by a virtually dry ditch. Furthermore, the major part of the electricity generated will accrue to Czechoslovakia from the Gabčíkovo dam, whereas the main purpose of the Nagymaros installation is to contain the surges from Gabčíkovo.

The latest plans, apparently, no longer demand that the Nagymaros dam should be in itself cost-effective. Hungary has no oil or coal that could be used to fuel peak-hour generators, and the only available

domestic fossil fuel, lignite, would, the government claims, be more harmful to the environment than would the dam.

Two years will be spent monitoring existing conditions of both river and ground-water before any overall movement of water is implemented, with a further year of monitoring once water movement starts. There are likely to be sewage works, and household and irrigation water for the affected areas.

One major objection to the project, however, remains unanswered: the threat to the flora and fauna of the Danube and its bayous due to the construction of the storage reservoir at Gabčíkovo and the diversion of some 25 km of its flow through an artificial concrete channel. Nor, specifically, have there been any assurances to the inhabitants of the settlements downstream from Gabčíkovo, who fear that, if the Czechoslovak dam were to be breached by an earthquake (the site is a seismic zone), there would be extensive flooding as far downstream as Budapest.

Public opinion in Hungary thus seems

Second front

WHILE pressing for the implementation of the Gabčíkovo-Nagymaros project, the Czechoslovak government has consistently castigated the Austrians for their "unilateral" decision to build a similar hydroelectric station at Hainburg, just upstream of the joint Austrian-Czechoslovak section of the river. The Hainburg dam was halted last winter under pressure from Austrian environmentalists, but the Czechoslovak government decided to take advantage of the friction to resume "active contacts" with Austria (after a gap of 25 years) on the use of the joint section. A protocol signed between the two sides in Vienna last week instructs a group of experts to formulate a programme, taking into account the "justified requirements and interests" of both states, the needs of international shipping and the environmental problems of the area, while making the fullest possible use of the hydroelectric potential of the river.

Vera Rich

unconvinced by the assurances of a government which, it believes, has been separately trying to withdraw from the project since 1981, but which has been unable to do more than buy time for its Czechoslovak partner. The clamp-down on media discussion of the project, early in 1984, was accompanied by what seemed to be carefully leaked rumours that Mr Kadar was trying to negotiate a Hungarian withdrawal by "quiet diplomacy".

The announcement that the project would after all go ahead has, perhaps unjustifiably, generated considerable disappointment.

Vera Rich

Estonian radioactivity

Lax handling of Soviet fuel

POOR safety precautions at a nuclear waste dump in Estonia have caused the death of at least one worker, according to an Estonian engineer who defected to Sweden last year. The defector, who had temporarily worked at the Saku nuclear dump some 15 km south-east of Tallinn, last week told a reporter from the *Sydsvenska Dagbladet* that the dump consists of a simple concrete bunker in the forest, which is leaking waste. Radioactive material is brought to the dump in trucks that are not properly equipped for the transport of hazardous cargo. Radiation-related disease is therefore common among those who have to service or transport material to the dump.

This is not the first time that reports of radiation sickness in Estonia have reached Sweden. Some three years ago, dissident Estonian sources claimed that it was endemic among the forced labourers employed to clean nuclear submarines at the Paldiski naval base.

Nuclear safety is an emotive issue in Estonia. For some years it has been thought that fuel for Finland's Soviet-built nuclear power stations is transported by rail to Tallinn and then shipped to Helsinki, and that spent fuel is returned by the same route, using routine facilities. The intensive civil defence training enforced in border areas of the Soviet Union means that every inhabitant of Estonia is constantly reminded of the dangers of radioactive contamination, while the presence of nuclear submarines at the Paldiski and Naissar bases is viewed by many Estonians as an infringement of the sovereignty of their republic. Four years ago, a group of human rights activists from Lithuania, Latvia and Estonia wrote an open letter to the heads of the governments of the Soviet Union, Iceland, Norway, Denmark, Sweden and Finland, petitioning that their planned Nordic Nuclear Free Zone should include the Baltic states; several of the signatories were duly convicted of anti-Soviet agitation.

Vera Rich



UK and proliferation

SIR—Your leading articles, on 27 June, 18 and 25 July and 1 August, on the proliferation and safeguards implications of emergent nuclear technology and nuclear commerce and on arms control difficulties, all relate to the current Non-Proliferation (NPT) Treaty Review Conference and its likely success or failure. It is a consolation that *Nature* has given this conference and its attendant issues so much coverage, when the other science journals and current affairs journals have barely afforded NPT a mention.

I should like to comment further on one particular aspect you raised on 1 August, that of the nature of Euratom safeguards as applied to the United Kingdom. There is a continuing problem in that the United Kingdom government still refuses to admit safeguards inspectors from the International Atomic Energy Agency or Euratom to the Windscale Magnox reprocessing plant at Sellafield, on the grounds that nuclear material from both British Nuclear Fuels' military designated plants and the electricity boards' reactors are either co-processed or processed sequentially. This means that the physical inspection of the fissile material within the reprocessing plant is not possible; only book-keeping checks are allowed. Foreign governments, including that of the United States, must rely upon the honesty of the British government when it states that no swapping of civil to military plutonium takes place. There are no meaningful safeguards applied to plutonium in the United Kingdom.

That this is becoming an important issue is spelled out by former UK government energy consultant Ian Smart in the August 1985 issue of *Nuclear Engineering International*, in which he points out in a preview article for the NPT Review Conference that "there is rising pressure on the United States, Britain and France to draw a clearer line between all civil and military nuclear operations, especially in the fuel cycle, for example, by renouncing any military use of material obtained by reprocessing civil reactor fuel. That pressure may increase in the aftermath of the [NPT] Review Conference."

The British government told Parliament on 13 May that it had no intention of raising the problems of applying safeguards at Sellafield at the NPT Review Conference. It is not surprising. In the 70 parliamentary questions it has been asked specifically on plutonium since the beginning of the year, it has been less than forthcoming. Indeed, so parsimonious has it been with details, that one begins to wonder whether it has anything insidious to hide. One contrasting example will suffice to show the problem at its starkest.

On 15 January, the Secretary of State for Energy stated to Parliament that it was "not the practice" of the government to

release details on the Mutual Defense Agreement on atomic energy matters signed between the United States and the United Kingdom. This agreement, dating from 1958, has enabled plutonium and highly enriched uranium and tritium to be exchanged across the Atlantic, as well as provided for other common nuclear weapons development. There are worries in some quarters that some of the plutonium sent from the United Kingdom was created in electricity board rather than military reactors, hence blurring the "atoms for peace" profile of nuclear energy.

On 23 May, however, when the government was asked for details of all the nuclear weapons tests carried out by or for the United Kingdom and the United States at the Nevada test site, their code names and whether conducted below or above ground, the government replied with full details, despite the fact that these tests were conducted under precisely the same 1958 Mutual Defense Agreement on which the government refuses to divulge details on fissile material exchanges.

To the outsider, it seems that something odd may be happening. It would be in the interests of all that the United Kingdom government should fully explain its position before or at the NPT Review Conference. The patience of the non-nuclear states over the antics of the nuclear weapons states aiding each others' armouries surely has only limited life before it snaps. Then we will witness the collapse of the non-proliferation regime.

DAVID LOWRY

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Down with metric

SIR—I agree with Dr D.C. Jolly (*Nature* 8 August, p.480) about the demerits of the metric system.

The metre is now defined as the distance travelled by light, *in vacuo*, in $1/299\,792\,458$ second. This seems to me an absurd number to choose — its only merit is that it preserves the metre as the basis of the SI system. If we were to start afresh, defining the speed of light instead of measuring it, we would surely choose a more convenient number such as 3×10^8 , or even better 10^9 . The latter would have the advantage that the new unit of length would be very close to the foot (about $1\frac{1}{2}$ per cent shorter) and this I feel opens the way to a rehabilitation of the pre-SI units.

The realization that light travels at almost exactly 10^9 ft s⁻¹ prompted me to investigate whether there is any system of units (metric, imperial or otherwise) that gives values close to integral powers of ten for the fundamental constants c , G and h

(or $\hbar$). Preliminary work on the rod-grain system was fairly encouraging but failed to find a sensible unit of time. However, when the strict requirement on units being powers of ten was relaxed to include *sensible* multipliers (i.e. not 3 and certainly not 2.99792458), it turns out that the foot-pound-second system works splendidly. In the relevant units, $c = 9.8 \times 10^8$, $G = 1.07 \times 10^{-9}$ and $4\hbar = 1.001 \times 10^{-32}$.

If we choose a system of units in which $c = 10^9$, $G = 10^{-9}$ and $4\hbar = 10^{-32}$ (all exactly), the units would be 1 new foot (or whatever) = 1.060 old feet, 1 new pound = 0.960 old pounds and 1 new second = 1.078 old seconds. I foresee no problems in introducing new feet and new pounds — we have coped remarkably well, all things considered, with the horrible metre and kilogram — but the new second may be something of a nuisance (clocks, calendars, etc.). However, the Earth's rotation is well known to be slowing down and the length of the day is increasing by roughly 8 seconds every million years. Thus, one day will contain 86,400 new seconds in about 800 million years' time. Perhaps we should delay the counter-revolution in our adopted system of units for a while.

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Pain in animals

SIR—The view expressed by Robert Sharp (*Nature* 25 July, p.290), that it is impossible to assess pain in laboratory animals, is not likely to be accepted by those who study this particular problem nor by those called upon to perform the task, including most members of the veterinary profession.

Although it may be said to be "impossible" to find guidelines for the assessment of pain that *guarantee* agreement on the degree of severity, guidelines *are* used and are being constantly improved. In most cases, in applying such guidelines, significant disagreement is unlikely, except perhaps on the distinction mild/moderate and moderate/severe pain, which may be resolved by taking the opinions of others experienced with the species involved.

In order to maintain and improve the health and welfare of animals and man, it continues to be necessary to subject some animals to experimental procedures. Unfortunately, it appears that those who are concerned about such use of animals are not always willing to accept that, despite the interferences sanctioned under licence as part of a procedure, the welfare of the animals concerned is and will remain, the first priority of those who carry out this work.

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Nuclear winter can cross Equator

A new calculation shows that smoke from nuclear conflagrations will reach the Southern Hemisphere from a northern nuclear war, especially in the northern summer. But many other problems persist.

How certain is the picture built up in the past few months of the nuclear winter that would follow a major exchange of nuclear weapons? Later this month, the group appointed by the Scientific Committee on Problems of the Environment (SCOPE) of the International Council of Scientific Unions to brood about the nuclear winter and the other consequences of nuclear war will be making public its first report; by all accounts, it will be more a judicious assessment of what remains to be done to define the uncertainties in the problem than a prescription of what the nuclear winter would be like, which is entirely appropriate.

Meanwhile, the article by Stanley L. Thompson on p. 35 of this issue will help to fill one important if small gap in previous arguments: smoke caused by a nuclear war between combatants in the Northern Hemisphere will rise in altitude faster, and spread more quickly to the Southern Hemisphere, than smoke from a nuclear war in the north during the northern winter. The conclusion is what would be expected from crude hand-waving; the explanation is largely that insolation energizes the atmosphere predominantly in the summer hemisphere. But since the interhemispheric transfer of smoke was of necessity neglected in the original calculations, and because its distribution affects the expected changes of temperature supposed to lead to nuclear winter, it is valuable that the point has been established by a strictly global three-dimensional model of the atmosphere.

Readers will also note that Thompson, like others working in this field, lists several points on which it appears that the first accounts of nuclear winter must in due course be refined. One of the most obvious of these is also one of the most inaccessible — the estimates that are used for the quantities of smoke lofted into the atmosphere after the conflagrations likely to be started by a general exchange of nuclear weapons. It is generally agreed that urban and rural fires will be respectively more and less effective generators of smoke, but there is much more scope for argument about the quantities likely to be produced by general urban conflagrations. One difficulty is that the issue cannot, by definition, be resolved by experiment; even conventionally started fires would be pale imitations of those likely to follow nuclear attacks on cities, while the few historical opportunities for observa-

tions (as at Dresden and Tokyo towards the end of the Second World War) have been lost. Moreover, to the extent that the severity of a nuclear winter would be directly determined by the amount of smoke released into the atmosphere in a nuclear exchange, the course of a post-attack winter is bound to be a sensitive function of the exact pattern of the attack.

Thompson also draws attention to the need for a better understanding of the way in which some part of the smoke generated by large fires would be promptly removed by the clouds they necessarily generate. This mechanism was part of the reason given by Dr Edward Teller last year for scaling down the original predictions of nuclear winter by R.P. Turco *et al.* (*Science* **222**, 1283; 1983). It would be surprising if large fires prove to be in some sense self-limiting in their consequences for direct insolation, but there is equally no obvious reason why effect and cause should be arithmetically proportional to each other. Again this is a point which is almost impossible to check directly.

Still another difficulty, which Thompson does not on this occasion list, is that of knowing what are the consequences of the inevitable patchiness in the cloud of smoke from several nuclear conflagrations, at least in the early stages in the evolution of a nuclear winter. Originally, Turco *et al.* supposed that several days would pass before their cloud of smoke was uniformly dispersed over some band of latitude. Put crudely, the difficulty is that of estimating the climatic consequences of two neighbouring vertical sections of the atmosphere, one normal, the other in which the vertical flux of solar radiation is much reduced by the effects of smoke in the upper troposphere.

Calculations cannot be straightforward, given that the system is far from equilibrium. But it is entirely possible that two such adjacent slices of the atmosphere would function as a means of carrying water vapour high into the atmosphere, so as to form clouds that would then remove smoke. But even if that were possible, precisely what happened would no doubt depend sensitively on the nature of the underlying terrain, sea surface or dry land.

Merely to list uncertainties such as these, among which are the difficulties of including real clouds rather than average cloudiness in climatic models, is not to demonstrate that nuclear winter is an illusion. At this stage, there is probably no

reason to dissent from the statement in Thompson's article that the uncertainties in calculating nuclear winter allow effects that range from "mild local cooling to sub-freezing temperatures on a global scale".

The immediate consequences of the likelihood that a major nuclear war, involving the use of a substantial fraction of the world's nuclear dedicated nuclear explosives, are similarly problematical. It has sometimes been argued that the possibility of nuclear winter of such a severity that most living things in at least one hemisphere would be killed argues for a reduction of nuclear stockpiles below whichever threshold may make such a catastrophe possible. But that is not a unique argument for arms control agreements that will effect a reduction of nuclear arsenals on such a scale; people's fond wish to sleep easily at night is a sufficient case for that.

Others say that the severity of the standard nuclear winter as first described by Turco *et al.* makes nonsense of existing arrangements for dealing with some of the consequences of nuclear war, plans for building fallout shelters, for example. In reality, the validity of this argument is more restricted. Even if the predictions of the kind of nuclear winter that would follow an all-out exchange of nuclear weapons are correct, the assumption that all nuclear wars must be all-out wars is surely mistaken. On at least some credible opinions about the course of events that might lead to nuclear war, the first steps would entail the use of small numbers of nuclear weapons, perhaps tactically in places such as central Europe. The function, misguided though it may be, of such hostile acts would be to extend a threat of seriousness to an opponent in time to force second thoughts, for which purpose just one or two weapons might suffice. Fallout shelters may have some value in those circumstances.

The implications of all this are simply that there is no obvious way in which the concept of nuclear winter affects strategy and international relations in ways that are unique. Non-nuclear weapons states such as India may rightly claim that the prospectus threatens them when they might expect to escape direct consequences of a major nuclear war between major nuclear powers, but that is only a part (and a recent part) of their traditionally stolid attitude on the need for strategic nuclear arms control.

John Maddox

Astrophysics

Plasma motion near comet cores

from D.J. Southwood

COMETARY fever is rising in the space science community with the launching of space probes to Comet Halley and the imminent encounter of the old ISEE 3 spacecraft (now renamed International Comet Explorer) with Giacobini – Zinner. One group could be said to have stolen a march: on 27 December last year the West German spacecraft of an international mission was enveloped by what was heralded as the first artificial comet, a cloud of ionized barium released from the spacecraft itself, and observed from Earth as well. But, by the time of the third meeting of the AMPTE (Active Magnetospheric Particle Tracer Explorers) science team held in June at Thornbridge Hall, Derbyshire, doubts were arising about the appropriateness of the cometary epithet amongst the scientists from the three nations (United States, West Germany and United Kingdom) involved in the mission.

It had, of course, been realized that the release, made in the solar wind on the dawnside of Earth one hundred thousand kilometres above the Earth's surface, would throw no light on the cosmogonic problems that could only be resolved by a spacecraft encounter with a comet; but it was confidently believed that the solar wind deflection, cometary plasma tail formation and depletion processes would be mimicked. The initial images obtained from the ground and from aircraft of the ion cloud expansion, elongation and eventual dispersal (after about five minutes), only served to confirm that prejudice. So did the magnetic field measurements made on the German IRM (Ion Release Module) spacecraft and its nearby companion, the UKS (United Kingdom Satellite), in the immediate vicinity of the cloud. In particular UKS recorded clearly the 'draping' of the field over the cloud exactly as was expected. Now, as the detailed analysis is proceeding, second thoughts are occurring. Analysis is complex because both IRM and UKS carried a complement of field and plasma instrumentation and the teams are having to come to terms not only with the images obtained from afar but also with the *in situ* measurements of local plasma conditions in and near the cloud.

The dramatic evidence that the team is now coming to terms with concerns the relative motion of the ion cloud and the faint residue of non-ionized barium that can just be detected in some of the images. Simple ideas from cometary theory led one to expect the cloud to move away from the Sun. The reason is straightforward: momentum is absorbed from the solar wind which is moving radially away

from the Sun. Gerhard Haerendel of the Max Planck Institut für Extraterrestrische Physik, the German project scientist, summarized the overall picture that is emerging. At the core is a very surprising result: the initial motion of the ion cloud formed at the core of the release was transverse to the solar wind direction. Where does the transverse momentum come from? It seems that it is due to a recoil effect from a stream of high speed ions leaking out of the cloud in the opposite sense. These ions later form the dominant component of the tail which ultimately extends thousands of kilometres away from the original hundred-kilometre-radius cloud at the core of the release. Although dissatisfaction had been expressed by many about the simple cometary theory based on magnetohydrodynamics (MHD), the high-speed ions were unexpected.

It is important to note that all interactions take place in a medium that is collision-free: the mean free path is orders of magnitude larger than the cloud. The electromagnetic field structure that produces the observed effects is complicated and presents an exciting challenge to plasma physics. In some respects, the physics resembles that required to describe collisionless shocks but with the further complication that the cloud 'obstacle' to the solar wind is relatively small and evidently three dimensional — whereas shock theory is normally developed for

planar structures. Shock scale lengths are short, however, and comparable with that of the cloud core, which is smaller than the magnetic (Larmor) gyration radii of either the solar wind ions (300 km) or the newly created barium ions (3,000 km). The ions behave as if they are unmagnetized. One consequence is that the obstacle has a high Hall conductivity, and therefore carries large currents perpendicular to any applied electric field. Such effects are negligible when MHD is valid. As often occurs in plasmas, it is when MHD breaks down that the really surprising physics is found. The momentum transfer between cloud and wind requires substantial electron heating as an intermediate process. Somehow a small fraction of the released ions manage to tap into the energy carried by the heated solar wind electrons and this gives rise to the curious effect reported.

Despite these comments, the results are probably still relevant to comets. Cometary cores can be small compared to the Larmor orbit scale of the massive ions believed to make up the cometary plasma environment. The relatively scant notice so far taken of such facts may be due to theory never before having to come to terms with the reality of *in situ* measurements. In the broader context the results on anomalous acceleration and heating of plasmas are going to be of importance not only to plasma physics but also for astrophysics. In these experiments we see a cosmic process writ small. The team have a second opportunity to test their ideas as another barium release took place outside the magnetosphere on the duskside of Earth on 18 July. □

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Mathematics

The duellist and the monster

from Ian Stewart

ONE of the more romantic figures in mathematics is the young revolutionary Evariste Galois, who wrote down his ideas on the theory of equations the night before a fatal duel, ostensibly over a lady. He introduced what is now called the Galois group of an equation, which measures how symmetrical it is and contains information about its solutions. Ever since, mathematicians have wondered which groups can occur as Galois groups. John G. Thompson (*J. Algebra* **89**, 437; 1984) has now found new ways to realize specific groups as Galois groups, including a remarkable 'sporadic simple group' known as the monster, because of its enormous size.

Galois showed that an equation can be solved by radicals — a formula involving nothing worse than n th roots — if, and only if, it has a restricted type of sym-

metry: its Galois group must be 'soluble'. For example, the general equation of the fifth degree has a Galois group containing all 120 different ways of permuting its five roots. This is insoluble, hence the quintic equation cannot be solved by radicals. The group is known to be insoluble because it contains within it a smaller 'simple' group A_5 with only 60 elements. (A simple group is one that cannot be broken down into smaller groups in a particular way.)

It seems to have been Emmy Noether, the most famous woman mathematician, who first asked which groups could occur as Galois groups. The most likely answer, "all of them", has never been proved or disproved. Over a period of time, various partial answers were obtained, notably by Igor Šafarevič, culminating in a proof that every soluble group is a Galois group. The

main obstructions to extending this result to all groups are the simple groups.

Simple groups are the 'atoms of symmetry'. Over the past few decades, an intense research programme has achieved the group-theoretical equivalent of Mendeleev's periodic table by finding the complete classification of all simple groups. These groups fall into many infinite families with similar properties, but there are 26 curious exceptions, the sporadic groups. The largest of these, the monster, was first placed in evidence by Bernd Fischer and finally captured by Robert Griess (who attempted, unsuccessfully, to rename it the friendly giant). It contains precisely 808,017,424,794,512,875,886,459,904,961,710,757,005,754,368,000,000,000 elements — roughly one septemdecillion symmetries. Griess tracked it down as a group of rotations in a 196,883-dimensional space. Many existence proofs for sporadic simple groups make essential use of computers, but the size of the monster seemed to exclude any computational approach. Griess solved the problem by not using a computer at all.

Modern algebra has recast Galois' original work in the context of 'algebraic number fields'. A field is a mathematical system in which all the usual operations of arithmetic can be performed, subject to the usual laws; so if every element of the field is a root of an equation with whole number coefficients, then we have an algebraic number field. A given field, K , can be extended to a larger one, L , by 'adjoining' the roots of an equation with coefficients in K . For example, the complex numbers arise from real numbers by adjoining a number i such that $i^2 + 1 = 0$. The Galois group of an equation with coefficients in K may then be thought of as the group of symmetries of L that fix K . Here, L is obtained from K by adjoining the roots of the chosen equation.

Thompson's work blends these two ingredients, Galois theory and simple groups, in a highly original way. It exploits a connection with a totally different area of mathematics — complex functions — that is becoming a recurrent theme in post-classification simple group theory. Thompson defines a notion of rigidity of a group (roughly, this means that its elements combine in a reasonably nice way), and proves that every rigid simple group is a Galois group of an equation with coefficients in a suitable algebraic number field. The monster may be proved rigid; and there are two alternatives for the algebraic number field. It can be either the rational numbers p/q (p, q are integers) or the extension obtained by adjoining $\sqrt{-71}$. Another family of rigid groups is $PSL_2(2^n)$, a group of projective transformations of a 2-dimensional space with coordinates in the finite field with 2^n elements (whose existence and uniqueness was also discovered, coincidentally, by Galois).

The function theory used goes back to

the classical era, and concerns 'Fuchsian groups'. These are certain groups of transformations of the complex plane, of the form $z \rightarrow (az+b)/(cz+d)$. Much is known about complex functions that are invariant under such transformations (for example, elliptic functions). Thompson's proof makes heavy use of a particular Fuchsian group, Γ , which has to satisfy a whole series of conditions: it is a torsion-free Fuchsian group of the first kind, of zero genus, with cusp number at least 6..., and so on. It is not necessary to define the terms to see that the choice of Γ must be made very carefully. As Thompson says, "It is a tight squeeze, but such a Γ exists". The equation whose Galois group is the given simple group is then constructed by an elaborate argument involving the Riemann surfaces associated with various subgroups of Γ . The combination of rigidity of the simple group, and the special features imposed on Γ , combine to make the strategy effective.

The above discussion may perhaps make Thompson's work seem like very specialized and technical mathematics, but its influence is likely to be extensive. The monster, for example, is acquiring

importance in mathematical physics. Symmetries in nature are central to much of quantum mechanics, so exceptionally symmetrical objects such as the monster stand out as being worthy of investigation. The realization of the monster as a Galois group has recently been applied by Jean-Pierre Serre (*Commentarii Mathematici Helvetici* 59, 651; 1984) to the Witt invariant of a quadratic form of importance in Galois cohomology (a modern descendant of Galois theory).

Thompson himself notes that further developments would be possible, given improvements in complex function theory. A 'better' choice of the group Γ would remove a rather artificial restriction in the current concept of rigidity. But even in its present form, the theory is a remarkable conjunction of group theory, Galois theory and function theory. It looks as if Emmy Noether's problem is starting to succumb to a three-pronged attack, and already the classification of simple groups is bearing fruit in several other areas of mathematics. □

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Sahel famine

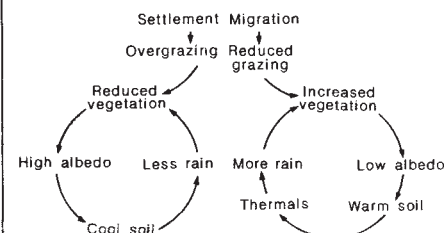
An ecological perspective

from John R. Krebs and Malcolm J. Coe

THANKS in no small part to the activities of Bob Geldof, public awareness and sympathy is probably greater than ever before for the plight of those living in the African Sahel, a semi-arid belt girdling Africa and including 10 countries from Senegal and Mauritania in the west to Ethiopia and Somalia in the east. The exact causes of the disaster are much less clear. While there is no doubt about the effect — 'desertification', that is loss of vegetation and replacement with bare soil, sand or rock — the factors that have brought this about are by no means obvious (Farmer, G. & Wigley, T.M.L. *Climatic Trends for Tropical Africa*; University of East Anglia, 1985). According to one view, probably shared by most of the public, the major cause is meteorological: a sequence of years in the 1970s with below average rainfall — below, that is, the average of 100 mm in the north and 500 mm in the south (Sanford, S. *Management of Pastoral Development in the Third World*; Wiley, New York, 1983). The contrasting idea is that to a large extent the desertification is man-made, resulting from a complex combination of demographic, cultural and agricultural changes exacerbated by ill-advised aid schemes and colonial development in the region (see figure; Cloudsley Thompson, J. *Int. J. Environ. Studies* 2, 35; 1971). This view has recently been argued forcefully by A.R.E. Sinclair and J.M. Fryxell in the *Canadian Journal*

of Zoology 63, 987; 1985. The issue is of crucial importance because, as we shall explain, it may have profound implications for the best ways of spending aid.

The key point of Sinclair and Fryxell's argument is that the present disaster was precipitated by a change in the lifestyle of the Sahel people, from being migratory pastoralists to settling down around water holes drilled by development agencies. They point to the fact that the migratory lifestyle is the traditional and effective way of coping with an arid environment for man and his cattle as it also is for many of the vast herds of ungulates that live in, and to the south, of the region. The 1.3 million wildebeest of the Serengeti Plains, for example, migrate seasonally from areas of high rainfall (1,000 mm per year) to semi-arid plains with a rainfall comparable to that of the Sahel (400 mm per year). These plains have a seasonal flush



Suggested sequence of the two climate-vegetation feedback systems in semi-arid regions (from Sinclair, A.R.E. & Fryxell, J.M. *Can. J. Zool.* 63, 987; 1985).

of green vegetation (especially annual grasses) after the rains, and it is at this time of year that the wildebeest move in to crop the nutritious young plants. The food is of such high quality that the animals calve during their stay in the semi-arid plains, but as soon as the surface water dries up they have to move back to the wetter areas where they feed on taller, perennial but lower-quality vegetation. The wildebeest's pattern of migration is seen in more or less parallel form in other herbivores, although some simply disperse rather than undertake regular migrations. It not only allows them efficiently to exploit their arid environment, but also sustains the vegetation. When the wildebeest leave the semi-arid plains, the annual grasses have a chance to grow (and perennial grasses to build up reserves) and set seed before moisture leaves the soil, providing the basis for next year's crop.

Traditionally, the pastoralists of the Sahel followed the same pattern as the wildebeest: taking or perhaps following their cattle northwards to calve, feeding on the high-quality grass in the most arid regions during the brief wet season, and then moving back south to keep their herds on low-quality vegetation during the rest of the year. An important factor bringing about the disruption of this traditional lifestyle was the provision of water at boreholes drilled in the semi-arid regions by development agencies. The wells had the effect of encouraging pastoralists to settle in the more arid parts of their range, with disastrous effects. Year-round grazing by their cattle deprived the annual and perennial grasses of a chance to recuperate and replenish themselves. Furthermore, the concentration of people and cattle around the water-holes compacted the soil so that plant establishment was suppressed. It is this effect, first noticed in the late 1960s before the supposed drought of the 1970s, that is central to the problem, according to Sinclair and Fryxell. The low rainfall of the 1970s probably exacerbated the problem but was not the primary cause; although there have been several years of below-average rainfall in the past 15 years, there were similar periods in the past that did not have comparable effects.

Other factors were also at work. The newly independent African states, for example, discouraged pastoralists from crossing national boundaries and encouraged them instead to settle near permanent water supplies. Meanwhile, medical and veterinary aid led to a rapid increase in population of both man and cattle, and traditional southern grazing areas of the pastoralists were turned over to crops such as sorghum, millet and peanuts.

Desertification of the Sahel, then, was brought about by gradual spreading and coalescing of denuded, overgrazed areas around boreholes. Sinclair and Fryxell go on to argue that the denudation may itself have longer-term effects on the climate —

a view that has been much discussed before and remains controversial. The essence of the argument is summarized in the figure. Loss of vegetation exposes the bare light-coloured soil, which has high reflectance and therefore has a cool surface that generates fewer upcurrents to carry moisture to higher altitudes where it can condense as rain. By contrast, in areas with better vegetation cover, the darker surface warms up to higher temperature, creating upcurrents that carry moisture (to some extent produced by the vegetation itself) upwards to form rain clouds. Thus overgrazing may conceivably cause a switch from one positive feedback loop to another, generating its own drought.

Sinclair and Fryxell share the view held by most commentators and fund raisers that food aid, while it must be our primary moral concern, it in itself not enough. Where they differ from the majority view is in the kind of long-term planning that is needed to prevent repeated famines. Their evidence leads to the view that the traditional migratory lifestyle evolved by man and beast for exploiting the semi-arid Sahel is the most efficient and most viable option. Drilling more water holes, building more houses and trying to encourage sedentary, crop-based agriculture in arid regions is not the solution. (Irrigation, another part of most proposals for development, has its own suite of problems and consequences, including salination of the soil, which are beyond the scope of this

article.) To re-establish the traditional mode of life of the Sahel people would be a long and difficult task: for one thing, the vegetation of the area would probably take 25 years to recover if given the chance; for another, the pastoral lifestyle could not be sustained at current levels of human and cattle populations. The inherent and enormous difficulties are not, however, a reason for ignoring the advice and thinking about how to act upon it.

On a more general note, the views of ecologists such as Sinclair and Fryxell, with many years of field experience and a grasp of the fundamental principles of ecosystem management, are surely important and should be part of any integrated long-term aid scheme. Similarly, the fundamental ecological research, by means of which such complex environmental issues can be thoroughly evaluated, should not be relegated to a sideline as it is, for example, by the current 'utilitarian' government view of university research in Britain. The really big research issues facing the world are not those relating to fractional increases in the industrial productivity of one or two already wealthy countries, but instead those concerned with the fragile relationship between man and his environment, so devastatingly portrayed by the Sahel. □

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Vision

Phototransduction changes focus

from David Attwell

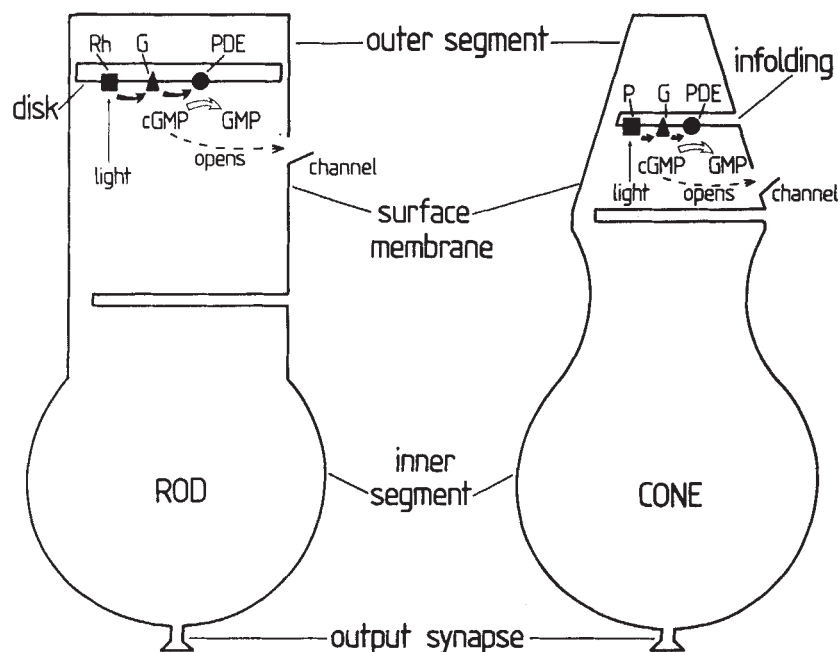
A MAJOR problem in vertebrate phototransduction has been solved. Patch-clamp experiments, published in *Nature* earlier this year¹⁻⁴ and in this issue^{5,6}, have resolved a long controversy over the identity of the so-called 'internal messenger', the signal that conveys to the surface membrane of the rod or cone the information that a photon has been absorbed by the receptor.

The need for an internal messenger was originally suggested for rods. Light is absorbed by rhodopsin located in membrane disks that are isolated electrically from the surface membrane of the cell. This results in the closing of ion channels in the surface membrane, and thus a change in the membrane potential of the cell. The first candidate for the internal messenger was calcium ions⁷, which were postulated to be released from the rhodopsin-containing disks and to diffuse to the surface membrane, where they would close the channels. The visual pigment in cones is located in infoldings of the surface membrane (see figure), rather than isolated disks, so that there is no absolute need for an internal messenger.

For simplicity, however, it was suggested⁷ that calcium played the same role in cones as in rods, with light allowing calcium to enter across the surface membrane of the cell and to close channels by binding to the inner surface of the membrane.

Biochemical and electrophysiological work on rods^{8,9} led to a challenge to the calcium hypothesis: light absorption was shown to result in activation (via a GTP-binding protein in the disks) of a disk-bound phosphodiesterase, which hydrolyses cyclic GMP. It was proposed that the resulting decrease in concentration of cGMP closed the ion channels in the surface membrane.

The first direct evidence that the agent acting on the surface membrane is indeed cGMP, and not calcium, came from Fesenko *et al.*¹ and Nakatani and Yau¹⁰. By perfusing different solutions on to the cytoplasmic surface of inside-out patches¹¹ pulled off rod outer segments, they showed that cGMP modulates the membrane current, while calcium does not. Further evidence for the lack of involvement of calcium came from whole-cell patch-clamp experiments³ which demon-



Schematic illustration of the main phototransduction pathway, which is now established in rods (left) and proposed for cones (right). In rods, light absorption by rhodopsin (Rh) in the rod outer segment leads to activation of a GTP-binding protein (G) that activates a phosphodiesterase (PDE). The PDE hydrolyses 3':5'-cyclic GMP to 5'-GMP, lowering the cGMP concentration. In the dark, cGMP keeps open surface membrane channels, through which an inward ionic current flows. Light absorption results in these channels closing, hyperpolarizing the rod's membrane, and thus generating a change in neurotransmitter release from the output synapse. In cones, cGMP opens the surface membrane channels^{5,6}. By analogy with rods, light absorption by the visual pigment (P, which has a structure different from rod rhodopsin) is proposed to lower the cGMP concentration via a GTP-binding protein and phosphodiesterase, although there is controversy about the existence of such a pathway in cones (see text).

strated that calcium-chelating buffers, introduced into the cell from the patch pipette, do not abolish the light response.

Now cGMP has also been shown to be the internal messenger in cones. Haynes and Yau⁵, using perfused inside-out patches of cone membrane, find that cGMP modulates the membrane current whereas calcium is ineffective. Reinforcing this result, Cobbs *et al.*⁶, who introduce cyclic nucleotides into whole-cell patch-clamped cones by including the nucleotides in the patch pipette, demonstrate that raising the concentration of cGMP in cones increases the light-modulated current. In both experiments, cAMP did not affect the current. This is important because biochemical work has suggested¹² that in cones light modulates the level of cAMP but not cGMP.

Where will studies in phototransduction go from here? The results of the patch-clamping experiments suggest three main approaches for future work. First, there are still large gaps in our understanding of cone phototransduction. Although cGMP modulates the cone membrane current, it has recently been claimed¹³ that cones lack the cGMP-phosphodiesterase, which one would expect (by analogy with the better-understood rods) to be a necessary intermediary between increases in illumination and decreases in the cytoplasmic concentration of cGMP. Further testing of this result would now seem essential. We also need to know what makes the cones less light-sensitive than rods: although

both absorb roughly the same number of photons, the change in membrane current produced by light is about 100 times smaller in cones than in rods. This is unlikely to be due to the light-modulated channels in cones carrying a much smaller current than those in rods as the channels in rods are among the smallest known, with a unitary conductance of about 0.1 pS¹⁴. A difference in gain at some earlier stage of transduction seems likely.

Second, attention will now focus on the molecular details of the interaction between cGMP and the light-modulated channels. Since, in both rods and cones, ATP is not needed for cGMP to open the channels, the cyclic nucleotide is presumably acting directly rather than through a protein kinase. The dose-response curve for the action of cGMP suggests that at least two cGMP molecules must bind to open each channel¹⁵. Unfortunately, the current flowing through a single light-modulated channel is too small to be resolved directly by present patch-clamp techniques, so it is not at present possible to assess directly the kinetics of interaction of cGMP with the channel, as has been done for the acetylcholine-gated channel at the neuromuscular junction¹⁵. Noise analysis of the cGMP-gated current offers one solution^{14,16}, and has led to the suggestion that cGMP increases the rate at which the channels open but has no effect on their closing rate¹⁴.

Lastly, now that the calcium versus cGMP debate can be considered closed

and the main pathway of events in phototransduction is established, interest is likely to grow in how the gain of phototransduction is regulated: gain changes occur in light adaptation, when the current response per absorbed photon decreases slowly during prolonged illumination. There are several points along the transduction pathway where gain control may occur. Light-induced phosphorylation of the visual pigment in rods might decrease the efficiency with which the pigment can activate the GTP-binding protein and hence the cGMP-phosphodiesterase¹⁷. The same phosphorylation also occurs in cones¹⁸. Could calcium have a role in the context of gain control? Recent work suggests that, rather than rising as required by the calcium hypothesis, the internal calcium concentration in rods drops when light is applied². Lowered calcium is known to decrease the light sensitivity of the cGMP-phosphodiesterase¹⁹ and to stimulate the guanylate cyclase converting GTP to cGMP²⁰, so the light-induced calcium decrease may well contribute to light adaptation. Other biochemical systems may also be involved: the suggestion that polyphosphoinositide hydrolysis plays a role in invertebrate phototransduction^{21,22} has been followed by reports that light alters polyphosphoinositide metabolism in rods²³, possibly affecting the rods' membrane potential²⁴. Sorting out the interactions of the different intracellular messenger systems is likely to prove exciting, not only because of the insight we will gain into the first stage of vision, but also because the light-activated events provide a model for other cellular control processes mediated by GTP-binding proteins. □

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Surface physics

Hopping atoms in crystal growth

from Colin J. Humphreys

How do crystals grow? This question has fascinated people ever since the beautiful six-fold symmetry of a snow-flake was observed through a ten-times magnifying lens. The paper by Bovin *et al.* on page 47 of this issue presents dramatic new information about crystal growth from images of growing gold crystals, magnified over 30 million times using a high resolution electron microscope and real-time video-recording.

At this magnification, individual columns of atoms in the gold crystals are clearly revealed; it appears that not only are atoms on the surfaces of small crystals in constant motion, hopping from site to site, but also that the crystals are surrounded by clouds of atoms in constant interchange with atoms on the crystal surface. The clouds of gold atoms extended up to 9 Å from the crystal surface, continually changing their shape and density.

Similar events have been observed independently by Sumio Iijima in Japan; a sequence of his micrographs showing very small gold crystals continually changing shape has already appeared in *Nature* (315, 628; 1985). Taken together, these observations may explain for the first time how atoms locate the most favourable positions during crystal growth.

Why have atom hopping and atom clouds not been observed before? The answer may be that so far most structural studies of crystal growth have involved techniques such as low-energy electron diffraction or X-ray diffraction, often using a synchrotron source. These techniques use relatively broad beams of electrons or X-rays, and the diffraction information is therefore averaged over a correspondingly large area of the specimen surface; local structural changes on a smaller scale than the incident-beam diameter cannot easily be detected. High resolution electron microscopy, on the other hand, produces images at atomic resolution, enabling atomic scale events on and above crystal surfaces to be detected.

Understanding how crystals grow is not only of scientific interest, but is also technologically important. Thus in the electronics industry, progress from very large scale integration to ultra large scale integration is constrained by the quality of the silicon crystals available, so that better knowledge of growth mechanisms in silicon and other semiconductors is essential. Similarly the behaviour of very small crystals is also of both scientific and technological interest. For example, many catalysts are composed of ultra-fine crystals whose activity is not well understood. The new ideas of atom hopping and atom clouds of Bovin *et al.* will have to be taken

into account in catalysis.

Pertinently the work of Iijima on ultra-fine particles is part of the Japanese government's Science and Technology Agency (STA) programme for Exploratory Research for Advanced Technology (ERATO), which specially supports materials research in areas selected for their intrinsic interest as well as strategic significance. Lack of British funds for this type of work was responsible for the emigration from Cambridge University to Arizona State University last summer of David Smith, a key member of the Bovin, Wallenberg and Smith team.

Finally, a word of caution about this elegant and important paper is in order.

Heisenberg, in his thought experiment to illustrate the Uncertainty Principle, argued that the position and momentum of a particle cannot simultaneously be measured precisely in an optical microscope because the incident photon beam interacts with the particle. To what extent, in the present work, does the interaction of the incident electron beam with the specimen influence the observed atom hopping and atom clouds, for example by momentum transfer and ionization? I believe that further experiments are needed to resolve this uncertainty, but the work reported is stimulating and may well have far-reaching scientific and technological implications. □

Colin J. Humphreys, has just moved from the Department of Metallurgy and Science of Materials, University of Oxford, Oxford OX1 3PH, to become Professor in the Department of Metallurgy and Materials Science, University of Liverpool, Liverpool L69 3BX, UK.

Transducing proteins

Yeast RAS and Tweedledee's logic

from Henry R. Bourne

THE discovery in yeast of genes with sequence homology to the *ras* oncogenes of mammals has raised the hope¹ that the sophisticated genetic technology available for the analysis of yeast physiology might become directly applicable to the biochemical analysis of mammalian tumorigenesis. It is known that the yeast RAS proteins are essential for proliferation and that they are responsible for the GTP-dependent activation of adenylate cyclase². Thus many have speculated that adenylate cyclase, which is implicated in a number of cellular signal-transduction systems, may also be the target of the p21 proteins encoded by viral *ras* oncogenes and their normal cellular counterparts. It has recently become clear, however, that this attractive notion is wrong. This does not mean that yeast experiments cannot help to define the role of p21 in regulating cell proliferation and causing malignant transformation: merely that they will do so less directly than had been hoped.

Two sets of observations had suggested a direct functional homology between yeast and mammalian p21. First, p21 from the cellular gene *c-Ha-ras* can substitute for yeast RAS proteins as a stimulator of adenylate cyclase and in support of yeast-cell proliferation³. The second observation involves a mutation that substitutes valine for a specific glycine residue and is believed to confer oncogenic properties on the mammalian *ras* protein. The same amino-acid substitution affects the presumptive GTP-binding site of the yeast RAS proteins, and causes them to stimulate yeast adenylate cyclase much more

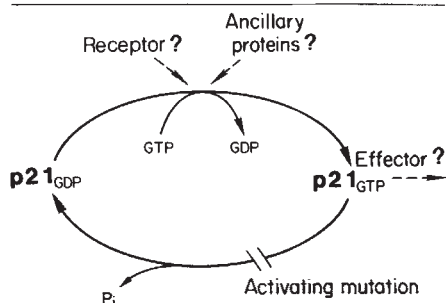
effectively, the consequent rise in cyclic AMP impairing the yeast cell's ability to stop proliferating when deprived of nutrition². The parallel with the oncogenic effects of the mutation in the animal protein is compelling.

All these observations had led to the supposition that yeast RAS might be functionally equivalent to the so-called G proteins that bind GTP and regulate the activity of adenylate cyclase in mammalian cells. But it is now clear from experiments *in vitro* that p21 does not in fact stimulate or inhibit mammalian adenylate cyclase. On page 71 of this issue⁴, Beckner *et al.* report studies of adenylate cyclase in membranes prepared from normal cells, from cells transformed by the viral oncogene *v-Ha-ras*, and from cells genetically deficient in the α subunit of G, the stimulatory GTP-binding regulatory protein of vertebrate adenylate cyclase. In all three cases, adenylate cyclase activity was unaffected by addition of relatively vast quantities of p21^{v-Ha-ras}, produced by expressing the oncogene in *Escherichia coli*. Because the *E. coli*-derived protein lacks a fatty acid modification that presumably facilitates membrane association of p21 in mammalian cells, Beckner *et al.* also tested smaller quantities of p21 (both *c-Ha-ras* and *v-Ha-ras*) purified from membranes of normal or *v-Ha-ras*-transformed mammalian cells, with the same result.

At the Cold Spring Harbor Symposium on Quantitative Biology in June, Michael Wigler reported a different kind of experiment: injection into frog oocytes of p21^{c-Ha-ras}, obtained by expression in *E. coli*, caused no change in cAMP. The negative

conclusion of this experiment was strengthened by a concomitant positive observation that the injected protein caused the oocytes to mature more rapidly. By comparison with p21^{c-Ha-ras}, mutant p21 'activated' for malignant transformation (by substitution of valine for glycine-12) proved much more potent in accelerating oocyte maturation, but also failed to alter cAMP synthesis.

In retrospect, earlier observations also indicated that the functions of yeast *RAS* and mammalian *ras* proteins would differ. For one thing, *ras*-induced malignant transformation reduces adenylate cyclase activity and cellular cAMP content⁵ — an effect in the opposite direction to that of yeast *RAS* proteins. Transformation by other, quite unrelated, oncogenes can also reduce cAMP. In any case, reduced cAMP is probably not a primary determinant of *ras*-induced malignant transformation, which is not reversed by metabolically effective cAMP analogues⁵. Tweedledee stated the position succinctly — "Contrariwise, if it was so, it might be; and if it were so, it would be; but as it isn't, it ain't. That's logic"⁶.



Speculative model of the biochemical role of p21 as a GTP-binding signal transducer analogous to the G-proteins. GTP-bound p21 stimulates an as yet unidentified effector enzyme analogous to adenylate cyclase or retinal phosphodiesterase. The p21 molecule becomes inactive when it hydrolyses bound GTP, a reaction slowed by activating mutations. Re-activation of p21 requires replacement of GDP by GTP, which is promoted by receptors (analogous to hormone receptors or photorhodopsin) and may depend on ancillary proteins analogous to the $\beta\gamma$ subunits of G-proteins.

Now that logic has prevailed, the original question of the function of the mammalian *ras* proteins persists. The best clues derive from homologies in amino-acid sequence⁷⁻¹⁰ and function^{2,11,12} among mammalian p21s, yeast *RAS* proteins, and a family of G-proteins that transduce extracellular signals across membranes of vertebrate cells. In addition to G_s , the G-proteins^{11,12} include retinal transducin, which transduces light signals from rhodopsin to a cyclic GMP phosphodiesterase, and G_i , the inhibitory regulatory protein of adenylate cyclase. (G_s and G_i are also termed N_i and N_o .) In the GTP-bound 'active' state, the G- and yeast *RAS* proteins stimulate the appropriate effector enzyme; each protein then hydrolyses the GTP to return to its inactive, GDP-bound state. In normal circumstances, it is

reactivated only when a signal arriving at a receptor molecule (hormone receptor or rhodopsin) induces the replacement of GDP by GTP. Manoeuvres that block the GTPase — hydrolysis-resistant GTP analogues or covalent modification of G, by cholera toxin — greatly enhance transduction of signals to the effector enzyme. It is thus significant that the *RAS*^{val19} mutation and the homologous substitution in the *c-Ha-ras* gene not only produce proteins with reduced GTPase activity, but also give rise to 'activated' phenotypes — including, in the yeast system, increased cyclic AMP synthesis². If the analogy between p21 and the G-protein family holds, p21 will be found to transduce information between signal receptor molecules and an effector enzyme (see figure). The search for these proteins is on.

As candidates for the signal receptor molecules, receptors for peptide growth factors are a good bet, although no convincing evidence for an interaction between such receptors and p21 has yet been reported. Because mammalian cells contain at least three distinct *ras* proteins, more than one effector enzyme may be discovered, in parallel with the multiple effectors regulated by the G-proteins. One possible effector for p21 is phospholipase-C (PLC)¹³. It has recently been shown that a GTP-binding protein participates in receptor-dependent stimulation of PLC, which catalyses production of inositol trisphosphate and diacylglycerol, putative intracellular messengers that may be involved in regulation of cell proliferation by peptide growth factors (see, for example, ref. 14).

The G-proteins are $\alpha\beta\gamma$ heterotrimers whose GTP-binding α subunits are most closely homologous to p21⁷⁻¹². Although unpublished experiments (A.G. Gilman, personal communication) have shown no

interaction of p21 with $\beta\gamma$ subunits derived from the G proteins, the possibility that p21 interacts with a $\beta\gamma$ -like component deserves further study. If such a component exists, its discovery might even speed the search for the putative effector and detector components. Indeed, the $\beta\gamma$ (rather than the α) subunit of one G-protein, G_{12} , mediates its regulation of the effector¹⁵, while the α subunit of another G-protein, transducin, cannot bind to its detector component, photorhodopsin, unless $\beta\gamma$ is also present¹⁶.

Finally, despite the unmasking of adenylate cyclase as an impostor for the effector enzyme of p21 in mammals, the yeast connection should not be forgotten. Further biochemical and genetic analysis of the yeast *RAS* system will probably identify other proteins, in addition to adenylate cyclase, that interact with the *RAS* proteins. Such yeast proteins could provide useful immunological and nucleic-acid probes for identifying the elusive proteins involved in p21-mediated signal transduction in mammals. □

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Organelle transport

A third front for cell motility

from Peter J. Hollenbeck

INTERNAL organelle movement is probably a universal feature of animal cells. Close scrutiny of cell types ranging from pigment cells to crawling fibroblasts to neurones reveals the constant transport of a variety of intracellular particles¹. How cells generate force for these movements is not known, but most studies of organelle movements have assumed that the mechanism is similar to that of either muscle or cilia, the two best-characterized mechanochemical systems. And the identification of the force-generating proteins of these two systems — myosin and dynein, respectively — in the cytoplasm has strengthened these expectations. But new studies using squid axonal cytoplasm suggest that a third force-generating system exists in cells which could be

responsible for microtubule-based movements of organelles^{2,3}.

Recent work from two groups has demonstrated that a soluble protein factor from the cytoplasm of squid giant axons can promote movements similar to organelle transport in the cell in a reconstituted *in vitro* system^{4,5}. Now two laboratories report progress towards the isolation and characterization of the protein motor which drives these movements. S.T. Brady², on page 73 of this issue, describes the partial purification of an enzyme with the appropriate characteristics for the transport motor. R.D. Vale, T.S. Reese and M.P. Sheetz³ report a further purification of a similar enzyme and demonstrate its ability to support the translocation of microtubules and the transport

of artificial organelles in an *in vitro* system. This protein, which Vale and co-workers have named 'kinesin', resembles neither myosin nor dynein in its enzymatic characteristics, subunit composition or interaction with microtubules.

The strategy for the purification of this putative transport motor originated from studies of reactivated organelle movements in extruded cytoplasm of squid giant axons. In the presence of ATP, these cytoplasmic preparations show organelle movements along microtubules very similar to those seen in the intact cell^{6,7}. Using such preparations, R.J. Lasek and S.T. Brady⁸ have shown that transport stops in the presence of a non-hydrolysable analogue of ATP, AMP-PNP, and that organelles become 'frozen' to the microtubules in a rigor-like state. The addition of excess ATP relaxes the rigor and restores the original organelle movements. Lasek and Brady explain these results by suggesting that the motor driving transport forms a stable complex with microtubules in the presence of AMP-PNP, which can be dissociated by ATP, in contrast to muscle and cilia, where AMP-PNP reduces rather than increases the affinity of the mechanochemical enzyme for its binding site. Based on this unusual microtubule binding behaviour of transported organelles, Lasek and Brady predict that the motile protein that powers organelle transport differs significantly from those driving muscle and cilia.

These predictions have been borne out by the findings both of Brady² and of Vale *et al.*³. To characterize the transport motor, both studies use microtubules as an affinity substrate, and select proteins from cytoplasmic extracts that bind microtubules in the presence of AMP-PNP and detach when ATP is added. By screening the products of their fractionation procedures for ability to generate *in vitro* translocation of microtubules, Vale *et al.* have succeeded in isolating a force-generating protein unlike dynein or myosin. Although it is also composed of several subunits, it differs from dynein and myosin in its relative molecular mass, its lack of the high relative molecular mass ATPase subunit and its response to enzyme inhibitors. One difference between the results of Brady² and of Vale *et al.*³ is that Brady obtains preparations with a rate of ATP hydrolysis comparable with other mechanochemical enzymes whereas Vale *et al.* demonstrate that force generation occurs in preparations with very low ATPase activity.

The force-generating properties of kinesin make the protein an excellent candidate for the motor that drives organelle transport in the nerve axon. Adsorbed to latex beads, the protein causes their translocation along isolated microtubules. It also increases the transport of native organelles along microtubules in a reconstituted *in vitro* system. An interesting feature of the bead movement is that

it seems to be unidirectional, whereas organelle movement in the intact axon is bidirectional. This could be an artefact of the *in vitro* system, could suggest that different mechanisms are responsible for movement in the two directions *in vivo* — a possibility suggested in earlier physiological studies. The question of exactly what kinesin is doing in the axon is clearly open.

Beyond the nerve axon, two exciting problems beg to be tackled. First, does kinesin represent a widespread class of force-generating proteins? Vale *et al.* have already found a protein analogous to the squid axonal translocation protein in bovine nervous tissue. But is kinesin present in other cell types that transport organelles along microtubules, and is it playing a role there? A second question is whether kinesin plays a role in types of motility other than organelle movement along microtubules. The fact that it generates force while bound to glass and to car-

boxylated beads suggests that any surface with a negative charge could provide a site for kinesin binding and be translocated relative to microtubules *in vivo*. In addition, Vale *et al.* have observed the movement of microtubules relative to one another in the presence of kinesin. It is possible that kinesin could support this type of microtubule-based movement *in vivo* — the mitotic spindle is an obvious candidate for examination. □

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Developmental biology

Drosophila proteins pave the way to gene mutations

from Philip Beachy

SEVERAL groups of developmental biologists with an interest in the fruit fly *Drosophila melanogaster* are currently engaged in what amounts to an experimental reversal of the information flow (from DNA to protein) specified by the 'central dogma' of molecular biology. The idea, as articulated by Fujita *et al.*¹, is to select a protein with interesting developmental properties, to isolate the gene encoding the protein and map it to a site in the chromosomes, and ultimately to test the gene's functional role by mutational studies. Such studies remain the most reliable test of a gene's importance in developmental processes, and a research group headed by Seymour Benzer at the California Institute of Technology has recently reached an advanced stage along this path.

Starting with a monoclonal antibody (MAb24B10) directed against a membrane-associated glycoprotein specific for photoreceptor cells, Zipursky *et al.*^{2,3} first affinity-purified sufficient quantities of the antigen (Ag24B10) to permit sequencing of 19 amino-terminal residues. Using synthetic oligonucleotides corresponding to a part of this sequence as hybridization probes, they then isolated a genomic DNA fragment that contains the amino acid coding sequence. By *in situ* hybridization to *Drosophila* salivary gland chromosomes, this coding sequence was mapped to 100B, a position near the tip of the right arm of chromosome 3. Several candidate mutations map within this region, one of which results in abnormal eye structure, but the exact positions of these

mutations are not known, and the identification of a specific lesion in the gene encoding Ag24B10 may yet require a considerable effort.

Some of the monoclonal antibodies (including MAb24B10) isolated by Benzer's group, although produced and initially screened using material from adult heads, bind to antigens in imaginal disks of the eye of third instar larvae. The figure, taken from Zipursky *et al.*², illustrates schematically the distribution of these antigens in the developing eye disk with respect to the morphogenetic furrow. As has been previously described⁴, this furrow sweeps across the disk in a posterior to anterior direction while, in its wake, epithelial cells differentiate and begin the patterning process that yields the clusters of photoreceptor and accessory cells that form the ommatidia of the adult eye. The immunofluorescent staining patterns illustrated in the figure are generated by a series of monoclonal antibodies whose cognate antigens act as markers for successive stages of eye disk development. Thus, Ag24B10, being expressed only on photoreceptor cells, lags substantially behind the pattern of MAb22C10, which stains neurones more generally. This in turn, lags slightly behind the pattern of MAb22G8, which stains the furrow. This temporal sequence presumably reflects the progressive commitment of cells behind the morphogenetic furrow, first to a neural fate and later to a more restricted pathway of differentiation into photoreceptor cells. Finally, with the appearance of the late pupal stage antigens, develop-

ment to a mature photoreceptor cell is complete.

From among the antigens of this series, 24B10 was chosen for further study because it is expressed exclusively on photoreceptor cells in both larva and adult at an early stage in these cells' maturation³. Until mutational studies of the 24B10 gene are performed, however, it may be impossible to say whether, in photoreceptor cell differentiation, expression of Ag24B10 is an early determinative event or a terminal response to one or more previous events. In this regard, the antigens bound by MAb3E1 and MAb22G8 are intriguing, as they may be involved in early patterning events at the furrow. Whatever the developmental roles of these antigens, the antibodies of this series serve as useful landmarks, and should add depth and precision to future studies of *Drosophila* eye development.

Using a similar approach, Wilcox, Brower and collaborators have characterized a set of cell surface antigens with intriguing distributions on developing *Drosophila* tissues⁹. In contrast to the eye disk series, monoclonal antibodies defining these antigens were selected and classified by their patterns of immunofluorescent staining on wing imaginal disks. The PS1 and PS2 (PS for position specificity) classes of antibodies stain complementary, non-overlapping portions of the late third-instar wing disk. A striking feature of the boundary between these two territories is its coincidence with the dorso-ventral lineage restriction (discussed in ref.10). PS3, a third class of antibody, stains the entire wing disk. Several lines of evidence, including coprecipitation of multiple components by each of the three antibody classes⁶ and cross-linking studies of intact cells⁷, indicate that the position-specific antigens on the wing disk are membrane-associated complexes and comprise a ubiquitously distributed component recognized by PS3 antibodies, and one set each of dorsally

(PS1) and ventrally (PS2) restricted variable components.

Restrictions in other imaginal tissues are observed. For example, on the eye disk, PS1 and PS2 are restricted primarily to regions anterior and posterior to the morphogenetic furrow, respectively. In general, however, these other boundaries are not as sharp and do not correlate perfectly with known morphogenetic events⁸. The possibility remains that classes of variable components exist whose distributions are not yet known. Indeed, by electrophoretic analysis of immunoprecipitates from other larval tissues and from earlier stages in development, Wilcox and Leptin⁹ have now identified several additional species of variable components. In the larval stages, the composition of these complexes in tissues such as gut, brain and salivary glands is highly characteristic for each tissue.

All these findings suggest that for any particular region of the developing individual, the properties of the cell surface that are important in tissue organization and morphogenesis may be specified by the unique combination of glycoprotein components expressed there. Speculations of this type must be tempered by the lack of any direct evidence pertinent to the role of these glycoproteins in development. As suggested by Wilcox and Leptin⁹, such a functional test could come from mutational studies, once the coding sequences are isolated and mapped.

Isolation of the genes encoding these proteins should resolve another issue: on the basis of correlated changes in mobility in several different gel systems and by comparison of partial proteolysis products, Wilcox *et al.*⁷ suggest that the wing disk variable components (and possibly the embryonic and other larval components as well⁹) are structurally related. Whether this relationship derives from the expression of a family of related genes, or from the modification of transcriptional and/or translational products of a single

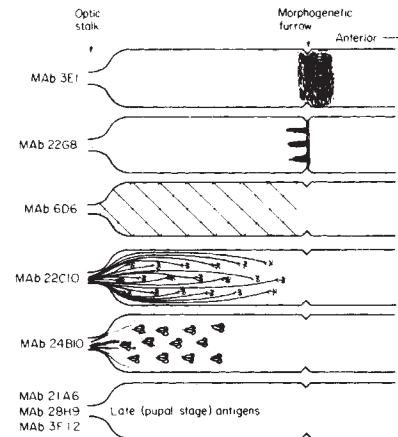


Diagram representing the sequential appearance of antigens during *Drosophila* eye development. Each strip represents a posterior-to-anterior slice of a late third instar eye disk. The staining patterns of the various monoclonal antibodies are schematically shown at the same stage of development, with reference to the morphogenetic furrow. As development proceeds, each pattern will progress to the right. (From ref. 2).

gene, should emerge from the characterization of the coding sequences and their RNA products.

Mutational studies provide the strongest causal link between a gene's products and a particular aspect of development. By such mutational studies, many *Drosophila* genes have been linked to segmentation, segmental specification and other aspects of pattern formation. In contrast to this genetically grounded approach, the research discussed here has employed monoclonal antibodies to identify proteins deemed interesting on the basis of properties such as their distribution within cells and tissues. This approach may be useful where something is known about the properties of proteins involved in a particular biological process. Unfortunately for the *Drosophila* developmental biologist, the confirmation of such a protein's importance in development by a direct genetic test lies only at the end of a long and arduous path. But in no other eukaryote of comparable developmental interest does such a path yet exist. □

100 years ago

DISINFECTION OF SEWERS

IN the last number of the *Lancet* (August 15, 1885) I have read of the measures taken by the Metropolitan Board of Works for the deodorising and disinfecting of London sewers. Between 30,000 to 40,000 tons of sodium manganate and from 10,000 to 12,000 tons of sulphuric acid are daily poured in the London sewers.

The oxidising and deodorising action of sodium manganate cannot be sufficient to prevent bacterial life, unless when the salt is present in large quantities. Considering the enormous volume of London sewage, it is not to be believed that even such a vast amount of manganate as 40,000 tons *per diem* would suffice to destroy bacterial life in the sewers. The adding of sulphuric acid to the manganate must certainly enhance the disinfecting action of the latter. Only, I do not understand why the quantity of sulphuric acid is relatively so small in comparison with the quantity of manganate. I do not see why manganate should be used at

all when sulphuric acid, a more powerful and less costly disinfectant, can be used alone.

It is not only during the fear of cholera invasions, but at all times, that I would wish the sewage to be *slightly* acidified with sulphuric acid. I am persuaded that this simple mode of disinfection would diminish considerably many infectious diseases. During the cholera epidemic of 1884, in Naples, I did my best to persuade the sanitary authorities of this mode of disinfection. My letters caused sulphuric acid to be used abundantly in the sewers and *pozzi neri* of Portici, Castellamare and Taranto; but this was done under pressure of approaching cholera, and abandoned as soon as the danger passed, no observation being made to measure the influence of the sanitary method adopted on local infectious disease.

Italo Gigliolo
Portici, August 20

From *Nature* 32 415, 3 September 1885.

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Solid-state physics

Switch as switch can

from H.L. Störmer

A NEW candidate has become prominent in the highly competitive race for the fastest switch in electronics, the record for which currently stands at about 10 picoseconds¹. While superconducting devices have been the undisputed leaders for many years, semiconductor devices have recently caught up and even surpassed them in speed performance. Superconducting devices remain unchallenged in their low power consumption, but require to be cooled close to absolute zero. Semiconductor switches, on the other hand, will function at ambient temperatures — though cooling improves their speed and power consumption.

Because each parent wanted to baptize its own child, the present winner runs under various pseudonyms (reviewed in ref. 2): high-electron-mobility transistor, two-dimensional electron gas field-effect transistor, modulation-doped field-effect transistor (MODFET) and selectively-doped semiconductor heterojunction transistor. The superiority of this device is based on a technique dubbed modulation-doping³, creating a thin (two-dimensional) sheet of highly-mobile electrons at the interface between two semiconductors. But its lead is short. Traditional silicon-metal-oxide-semiconductor field-effect transistors (Si-MOSFET) are close behind, carrying with them the weight of a mature technology. And new devices are on the horizon, the heterojunction bipolar transistor⁴ and the metal-base transistor⁵ being recent contenders.

On this busy stage, a new actor has now appeared: the velocity modulated transistor (VMT). Its advocates promise a sub-picosecond electrical response, and a recent paper by K. Hirakawa, H. Sakaki, and J. Yoshino⁶ contains experimental data substantiating one of its ingredients. The basic idea is simple and has been stated in various disguises, but the recent publication pushes it into the limelight.

Virtually all semiconductor switches are transistors. These are three-terminal devices characterized by an in-terminal, an out-terminal and a control-gate in between, which can enhance or suppress the flow of carriers from in to out. The gating action is provided electrically by applying a voltage or a current to the gate terminal. The gate controls the conductivity σ of the semiconductor, where $\sigma = ne\mu$. Here n is the density of carriers in the material, e is the electronic charge and μ is the mobility. In general, variations in σ can be induced by a variation of the carrier density n , or by a variation of their mobility μ . All traditional transistors are of the first kind: the predominant action of the control gate is to vary n , though invariably μ is also

affected. The new proposal is to attack the carrier mobility μ , leaving n fixed. Since, for a given electric field, the mobility is proportional to the average carrier velocity, the effect is termed velocity modulation. What is the advantage over density modulation?

Density variations require carriers to be pumped in and out of the system. Carriers have to be displaced over distances as large as the dimension of the device. This requires time. Modulation of the carrier velocity is subject to fewer delays and, hence, the device speed can be considerably enhanced. How does one change the velocity of the carriers? Following Sakaki⁷, the device is constructed in such a way that the action of the control gate is to move the carriers from a low-loss region into a high-loss region. Increased carrier scattering will reduce their average velocity and produce the desired effect. The actual distance that carriers will have to travel from the high-mobility to the low-mobility region can, in principle, be kept exceedingly small^{7,8}.

Hirakawa *et al.* use a traditional GaAs-(AlGa)As MODFET to establish the principle of the VMT. Field effect transistors are basically parallel plate capacitors of capacitance C embedded in a semiconductor. Conduction along one of the plates (channel) is controlled by a bias voltage ΔV applied to the opposing plate (gate electrode). The simple capacitor equation predicts a carrier density variation of $\Delta n = \Delta VC/e$ and, hence an associated variation of the conductivity $\sigma = ne\mu$ in the channel. Operated in this way a MODFET represents a traditional 'density-modulated transistor'.

For their demonstration, the authors used an additional feature of this device. In a MODFET, the channel consists of a low-density ($n \approx 10^{12} \text{ cm}^{-2}$) two-dimensional carrier system confined to the interface between two semiconductors, GaAs and (AlGa)As. The metal gate electrode covers the opposite side (front side) of the thin (AlGa)As layer. The carriers reside on the GaAs side of the interface being pulled against it by electrostatic forces. Carriers are free to move along the interface and are quantum-mechanically confined in the perpendicular direction. Since the (AlGa)As is heavily doped with impurities, close proximity of the carriers to the interface increases carrier scattering, while further separation from the (AlGa)As material reduces it. The distance between carriers and interface can be controlled by a second electrode placed on the GaAs side (back) of the structure⁹. Its effect is to push the carrier more or less strongly towards the scatterers.

Applying a combination of voltages to back-gate and front-gate, the average carrier mobility can be varied while the carrier concentration in the channel remains fixed. A similar arrangement (substrate bias) is commonly used to achieve this effect in Si-MOSFETs¹⁰ but the mobility variations are not very strong. In the MODFET structure, Hirakawa *et al.* were able to modulate the conductivity of the channel by up to 56 per cent without actually changing the carrier density. To achieve this control, the carriers had to move by less than $0.01 \mu\text{m}$ towards the interface, compared with approximately $1 \mu\text{m}$ in typical state-of-the-art density-modulated transistors. Hirakawa *et al.* postulate switching times as short as sub-picoseconds, a decrease by more than a factor of ten over the fastest present devices. But, for the time being, this is not substantiated.

Will it work? No fundamental law of physics forbids it, but at this juncture I see several significant hurdles. A 56 per cent conductivity variation is sufficient for initial demonstration purposes, but engineers would eventually want to see a 100 per cent variation. This would require some very strong scatterers — strong enough to bring the current essentially to a stop. At present it is difficult to imagine this. A second electrode (back-gate) adds considerable complexity, in particular since it has to be buried under the device to bring gate voltages to an acceptable level. The present experiments were performed with very small source-drain electric fields where the concept of mobility is justified. Fast devices function at exceedingly high fields where this concept fails and a constant carrier velocity is approached. Can this saturated drift velocity be modulated in a similar way? It is hard to see a mechanism. In general it seems that as devices become smaller and smaller, and approach the region where carriers in a transistor experience only a few scattering events on their way through the channel, a sensible separation between 'density-modulation' and 'velocity modulation' will eventually disappear.

In one picosecond, light travels $300 \mu\text{m}$, which is the distance a signal travels within one switching cycle of such a device. Sit back and think how you would design a computer with such blinding speeds. □

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Ozone, dust, smoke and humidity in nuclear winter

SIR—We wish to comment on recent correspondence on nuclear winter. Brown¹ draws attention to the Tunguska meteor explosion of 1908, which was estimated through aerodynamic calculations to have generated up to 30 million tons of NO, similar to the amount generated by about 6,000 megatons of nuclear explosives². But there are several reasons why the Tunguska event may not be useful in calibrating the effects of a major nuclear exchange. Recent ice core analyses of NO_x deposition from 1908–11 suggest that the NO production by the Tunguska meteor was equivalent only to 600 megatons or less of explosives³. Although ozone variations deduced from solar observations during the same period range as high as 20 to 30%, the ozone effect is uncertain because of statistical noise in the data base⁴, so that a significant cause/effect relationship cannot be established.

Brown¹ and Peczkis⁵ both confuse the relationship between the optical depth of an aerosol cloud, the composition of the cloud, and its effect on sunlight intensity and climate. Volcanic clouds are composed of dust-like aerosols that scatter light efficiently but absorb very little. The recent eruption of the El Chichón volcano (Mexico, April 1982) produced a hemispheric cloud with a measured optical depth of about 0.3 at its zenith; although the cloud reduced the direct solar beam by about 25%, the forward-scattered light made up for most of this with a net loss of intensity of only 2 to 3%⁶. Smoke aerosol, by contrast, efficiently absorbs light, so that the same optical depth of smoke (0.3) may reduce the intensity of sunlight by a full 25% at high noon (and by more than 25% at other times of the day). It follows that smoke injected at high altitudes can be much more efficient than dust (per unit optical depth, or unit mass) at inducing climatic change⁶.

Peczkis⁵ has misinterpreted Stothers⁷ data on the Tambora eruption of 1815⁷, confusing scattering with absorption, and thus calculating a 90% depletion of solar insolation. The volcanic cloud may have reduced the direct solar intensity by that amount, but the net solar intensity would have been reduced by less than 5 to 10%. In fact, Stothers' estimate of the volcanic aerosol optical depth, which is larger than previous estimates, implies an average global temperature decrease of about 2°C (see ref. 8), while the records show a roughly 1°C decline⁶. Even so, the year following the Tambora eruption became famous as the "year without a summer", illustrating the potential climatic significance of even small average temperature fluctuations.

Historical fires would provide useful calibration points for nuclear winter calculations if sufficient data were available.

In most cases, there are difficulties in determining the quantity of material burned and the height of injection of the smoke. While German and Japanese cities were extensively burned during the Second World War, scientific record keeping was scanty and little can now be deduced quantitatively. And, although natural wildfires often burn for weeks or months affecting 10,000 km² or more⁹, this situation differs markedly from the aftermath of a nuclear war, when hundreds of thousands of square kilometres of wildlands and hundreds of cities might burn in a matter of days.

Many of Peczkis' incidental statements about fires and smoke require qualification. For example, cooling is not expected beneath thin low-level smoke palls generated by persistent smouldering wildfires¹⁰, although significant cooling has been observed below high-level smoke clouds¹¹, supporting the prediction of possible quick cooling and freezing under thick nuclear clouds, at least on a regional scale¹². Moreover, smoke does not have to be injected into the stratosphere to cause a nuclear winter¹³; injection into the middle and upper troposphere is sufficient¹⁴.

Almost all observations point to rapid geographical dispersion of smoke plumes from large fires¹⁵, which is why Peczkis' speculation that such dispersal may not occur in nuclear fires is puzzling. Moreover, his estimates of smoke emissions in past forest fires are too high; typical forest fuel loadings are $\approx 2 \text{ g cm}^{-2}$, and one-third or less of this fuel generally burns in intense fires^{10,16}, so that less than 5 million tons of smoke should have been generated by even the largest historical fire complexes. The strongest effects of such fires would be expected to be limited to nearby regions¹⁷, but even so, further analysis of meteorological and geographical records may be warranted.

Katz¹⁸ is wrong in stating that water condensation and smoke scavenging processes have been ignored in published nuclear winter calculations; both prompt and delayed washout of smoke have been included^{10,13,16}. Katz is most concerned with the moisture drawn into a fire by the convective winds established over the heat source. Excess humidity may be efficiently wrung from a humid smoke column in the form of local precipitation, as in the "black rain" which fell on Hiroshima on 6 August 1945¹⁹, although smoke is apparently much less efficiently removed^{10,20}. The condensed water that does not precipitate from the fire column disperses with the smoke clouds and soon evaporates — as do cumulonimbus anvils — releasing any smoke previously scavenged.

The temperatures of stabilized smoke clouds are sub-freezing when the altitudes attained are greater than a few kilometres. The water content of the clouds should also be rather modest because of the de-

hydrating effect of the "cold trap" over the smoke column, through which most of the smoky air must initially pass. Hence, a smoke cloud downwind of a fire is not likely to precipitate spontaneously, even if cooled further. The humidity of such a cloud is soon dominated by the humidity of the background air which dilutes the cloud, and by the absorption of solar radiation, which can heat and stabilize the smoke layers against convective penetration and washout^{10,12–14,16}.

Water condensing in the chilled slabs of air beneath a dense cloud of smoke may form a fog on any smoke particles that happen to be present, but this effect is of minor importance for a variety of reasons: (1) significant temperature reductions must first occur to trigger the effect; (2) the smoke at such low altitudes does not have a major role in nuclear winter climate effects; (3) the condensation is essentially a one-time process whose smoke removal efficiency may not be as high as Katz implies^{10,20}.

Idso's comments²¹ on the nuclear winter concept are muddled. Physical scientists certainly should favour partially calibrated models over uncalibrated ones in analysing potential threats to the global environment. If a grand experiment has not been done, or cannot be done, prognostication with the best models available is a necessity. Studies of volcanic explosions, meteor impacts and dust storms provide fundamental insights into the responses of the Earth's climate system to heavy aerosol loading, and contribute to a clearer understanding of the nuclear winter phenomenon. The martian atmosphere is not such a pure vacuum as to preclude wind-driven dust storms there of global scale, which provide a unique opportunity to investigate planet-wide anomalies in atmospheric dynamics and climate triggered by clouds of soil particles.

We thank Steven Soter for helpful comments.

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Visual perception as a text

SIR—In his recent review of Bruce and Green's new textbook¹ on visual perception, Sutherland² first criticizes the authors for their attention to the ideas of the late J.J. Gibson and then holds a number of these ideas up for ridicule. We maintain that Gibson's work belongs in textbooks and that Sutherland's attacks on particular concepts are irresponsible.

Bruce and Green stress, in Sutherland's own words, "... some of the exciting new developments that have taken place over the last 20 years". Gibson's work clearly meets this criterion. It challenges many long held assumptions about vision and is generating a considerable amount of research. The acclaim of a number of eminent writers³⁻⁵ suggests the challenges are serious. Also one of the textbook's stated concerns is the ecology of vision. Since Gibson's ecological optics constitutes the only sustained attempt to bring ecological concerns into the heart of perceptual theorizing (albeit not the only attempt to connect ecology and perception), the authors would have been irresponsible to omit Gibson's work.

Sutherland's comments about particular concepts show that he does not understand Gibson's ideas. First, Gibson's claim that information in the optic array can be picked up without computational or inferential processes is said to be "... so silly that it is not worth taking seriously". Second, it is said that "Gibson was of course correct in believing that the starting point for what we see is the visual image, but who has ever doubted that?" The trouble here is that Gibson is one of the few who did doubt "that", so Sutherland has Gibson backwards. Moreover, Gibson's alternative to traditional image concepts, the optic array, is essential to the meaning and plausibility of his claims that perception is not inferential or computational. If one accepts "optical images", retinal images, or the like as starting points for visual perception, then the process *must* be inferential or computational. Sutherland is being consistent within his own views. He and those whose ideas he champions (Marr) accept the received views of the "givens" and concentrate their considerable cleverness on

computational innovations. What Sutherland fails to recognize is that Gibson's account begins prior to those he is accustomed to. Gibson began by reexamining the nature of the givens for perception and the task of perceiving itself⁶.

Finally, it is stated that one does not need to read Gibson to understand that time to collision is specified by variables in optical flow. We might add that one need not read Newton to understand that action equals reaction in the play of physical forces. Gibson brought the basis for time to collision analysis into psychology⁷ and his intellectual heirs continue to study its implications^{8,9}.

By turning Gibson's ideas on their head Sutherland showed that he has not taken them seriously enough to find out what they are. The point of a textbook, such as Bruce and Green's, is to allow students the luxury of discovering the ideas that exist *before* evaluating them.

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X chromosomes and dosage compensation

SIR—D.A. Smith suggests that the primary role of dosage compensation is to equalize the level of transcription between X and autosomes in males rather than to equalize X-chromosome transcription between males and females (*Nature* 315, 103; 1985). He derives this hypothesis as the logical corollary of the extremely deleterious effects of whole-chromosome aneuploidy. For mammals, X-chromosome inactivation would then be a secondary phenomenon necessary to prevent excess X-linked gene activity in females.

I would like to raise two questions. The first relates to the logic of the argument. I agree with Smith's point that, in the evolution of chromosomal sex-determination, the loss of an X chromosome in males must have forced these biological systems to deal with the problem of ensuing aneuploidy. But the solution to that problem need not have been an enhancement of X-linked activity in males. It is conceivable that the matter was resolved by gradual and individual evolution of the sex-linked genes and the system as a whole, so

that lower levels of these products would be adequate. If this were the case, one would still need to inactivate one X in mammalian females to avoid functional hyperploidy but one would not find any traces of a "class of genetic elements which function to modify X-chromosome expression" in males.

The second point deals with the feasibility of testing Smith's hypothesis experimentally. I do not see how one can determine that the level of transcription of X-linked genes is equivalent to that of autosomal genes. How can comparisons be made given that we can only study individual, and therefore necessarily different, genes? For example, what is the rate of transcription for hypoxanthine-guanine phosphoribosyl transferase or factor VIII that is equivalent to that for galactose-1-phosphate-uridyl transferase or factor IX? Answers to these questions would imply that there are global rates of transcription; something that is certainly well worth investigating but that is not established.

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DNA topoisomerases in eukaryotes

SIR—In a recent *News and Views* article (*Nature* 316, 394-395), G. North provides an interesting and informative summary of recent research on eukaryotic DNA topoisomerases. We were, of course, pleased to see our own work, which had been presented at the Cold Spring Harbor meeting on Chromosome Structure and Expression (8-12, May 1985), cited in the article. However, we feel that our results and their implications were not described with adequate accuracy. North says we have shown that "supercoiled plasmids are relaxed in yeast so long as one of the *TOP1* or *TOP2* genes is functional, which implies that topologically constrained DNA in yeast will be maintained in a relaxed state". What we have in fact shown, more accurately stated, is that the bulk of supercoiled plasmids are relaxed, with a half-life of several minutes, provided that one of the two topoisomerase genes is functional. Thus our results do not exclude the possibilities that there may be a very small population of plasmids which is maintained in a torsionally stressed state and that the bulk plasmid population may undergo transient torsional stress. Given these caveats, there may not be as great a difference between yeasts and higher organisms as North implied.

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Obeisance to the camera

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Framing Science: The Making of a BBC Documentary. By Roger Silverstone.

British Film Institute, 81 Dean Street, London W1V 6AA, UK/University of Illinois Press: 1985. Pp. 239. Hbk £16, \$29.95; pbk £7.95, \$14.95.

MANY telling remarks spatter this account of how a television programme in the BBC's *Horizon* science series was put together. One that will strike home with scientists occurs at the International Rice Research Institute (IRRI) in the Philippines. The producer, Martin Freeth, has spent the previous week filming farmers in that unhappy country, as they bring in a meagre harvest. Now he and the crew are in the luxury of the Western-style laboratory at Los Baños. Roger Silverstone records:

It is interesting to note the way in which the IRRI scientists, whether or not they are aware of Martin's intentions in filming them, and whatever their anxieties, accept without demur his instructions, his direction, in a way no different from the peasants of the Cagayan. Filming has that capacity: to turn all its subjects into peasants.

Freeth's intentions were a little devious. Although he was making a science documentary, he did not mean to involve himself or his audience in any painstaking explanation of the plant breeding work at IRRI. He was shooting "wallpaper", a technical term for impressionistic shots that can be used in developing a line of argument. He wanted images of advanced research that would contrast ironically with the poverty he had just been filming. The message of the programme was that the much-vaunted plant breeding programmes of the Green Revolution had failed to help the poor.

Some researchers at Los Baños understood and connived in Freeth's purposes; others were suspicious and defensive. All finished up as film actors doing what they were told to do. While the camera is turning, the producer-director is god. But playing god is an anxious task, and Silverstone's description of Freeth's torments, in the midst of never-ending difficulties and adventures, give the book some of the qualities of a first-rate novel. It deserves to become a classic, whether counted in the literature of movie-making or the sociology of the media.

Silverstone is a sociology lecturer at Brunel University, UK, and he obtained the BBC's permission to attach himself to a producer for the period of making a film. He wanted to find out how one goes about the task of explaining a complicated subject to the general public, within the *Horizon* series. (*Horizon* provides the backbone of the BBC's science output, and many of its programmes are shown in the *Nova* series in the United States.) In this

respect, Silverstone was to be disappointed, because the eventual product was no mainstream science documentary. More than making up for that was his good fortune in finding, in Freeth, a producer who not only tolerated the sociologist's presence but was able to articulate the tensions between scientific curiosity and



In the field — the BBC crew on location in the Cagayan, Philippines.

social conscience, between the ideal and the expedient, between argument and art.

The art took a beating when Freeth was banned from further filming in the Philippines, after another BBC programme had criticized the Marcos regime, and his idea of showing the agronomy of poverty from the viewpoint of a family in the Cagayan had to be abandoned. In the end the film presented a global thesis of a kind that Freeth had tried to avoid, with the Philippines looming relatively small in a programme that also took in Mexico and Bangladesh. It even had to be rounded out with library footage. Strangely, however, in the final phase of editing Freeth was agreeably surprised to be told by the high-ups in the BBC to strengthen the film's political message about the stark choice between a real Green Revolution and a Red Revolution among the world's agrarian poor. From Silverstone's account, this was clearly a professional rather than a

political judgement: if the film was to work, as entertainment and information, its argument had to be made clearer.

When *A New Green Revolution?* finally went out, in January 1984, after far more time and money had been spent on it than was ever intended, it lacked the polish of Freeth's other documentaries, such as *Einstein's Universe* and *The Other Kenya*. It was, though, a hard-hitting film, telling a shocking story, and it was praised by television critics. But what about the scientists, who gave so freely of their time and advice, or performed obediently in front of the camera? Is it worse to finish up on the cutting-room floor, or to be used as evidence in a programme that does not exactly condemn science but shows a wide gap between promise and performance?

Silverstone sent a questionnaire to the scientists whom Freeth had consulted, asking them what they thought of the programme. Several of them were critical about the off-hand way in which it dealt with the substantive science, and they noted political bias, even though they generally agreed with the overall thrust of the argument about science in the Third World. Other biologists who saw the programme felt the same way, and were defensive on behalf of their plant breeding colleagues. Anyone who expected an exposition of the technical wonders of modern agrobiotechnology was bound to be disappointed.

When challenged by the General Advisory Committee of the BBC to explain what scientific advice he had taken, Freeth was able to cite as his chief authority Keith Griffin, economist and President of Magdalen College, Oxford. BBC producers are not supposed to air their own political views. In Griffin, Freeth had found an expert spokesman for the thesis of the programme, although he was brought in more than a year after filming began.

Disgruntled plant breeders should read Silverstone's book to understand how Freeth approached his task. Anyone involved in making a television programme about a serious subject is walking through a minefield of conflicting expert opinions. He must either give every point of view and finish up saying nothing, or else decide whom to believe and face criticism from the others. This book records that Freeth made his choice only after much heart searching, and he never forgot that he was making a science documentary. His track record was such that the BBC supported him throughout.

What general conclusions are to be drawn about science on television from this atypical example? Silverstone himself notes the enormous effort that goes into documentary production, and the extent to which producers have to rely on serendipity, but wonders whether the BBC would have allowed as much freedom to Freeth, about the politics of the program-

me, if the subject matter had been nearer home. Who can but share this doubt, after the recent row about censorship concerning Northern Ireland?

Given the undoubted freshness and originality of *A New Green Revolution?*, the lesson, I think, for the BBC, Channel 4, WGBH, WNET and other organizations handling science on television, should be "stay loose". Planners find it convenient to have slots such as *Horizon*, *QED*, *Tomorrow's World*, *Nova* and *Nature* which take care of so many hours' broadcasting each year, but there should be no insistence on a standard product, no prejudices about how individual programmes ought to be made.

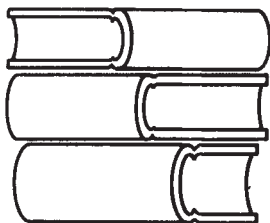
Open-mindedness about treatment helps to make sure that "soft" issues are not dealt with too softly. It is equally important in tackling the trickiest aspects of "hard" science in the most palatable fashion, as Freeth himself demonstrated in dealing with general relativity. Prime-time television has to be, first and last, a form of entertainment, competing with sport and drama. Only through the skill of programme makers who endlessly refresh the formats can serious science expect to pass that test. Along with all the other peasants, scientists and programme controllers have no choice but to trust the producer. □

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Thought on crystal shapes and sizes

David Goodman

The Scientific Reinterpretation of Form.

By Norma E. Emerton. *Cornell University Press*: 1985. Pp.318. \$29.95 (North America), \$32.95 (elsewhere).

BIOGRAPHIES and histories of ideas have been the most commonly treated subjects in the history of science. Each has its pitfalls. A biography threatens to distort the judgement of the author, who is liable to attribute unwarranted significance to the dates of birth and death of his subject, and to see a period of several decades solely from the viewpoint of the individual he is studying. In the history of an idea, fragments of a natural philosopher's writing may be taken out of context because they happen to touch on the idea under study; superficial similarities may be discerned in the thought of men living centuries apart; and scientists of very different periods may be presented as consciously contributing to the progressive development and eventual culmination of a particular theory.

Most of these distortions are avoided by Dr Emerton in her study of the development of the concept of form—the idea, so prominent in the history of science, which sought to relate the perceptible properties of things to some internal nature. Her analysis is careful and accurate, and the theories of individual natural philosophers are kept in context, though she succumbs to anachronism in her comment that the geometrical treatment of form by the fourteenth-century philosopher Nicole Oresme "looks towards" seventeenth-century corpuscularianism "for its fulfilment" (p.97). Although concentrated attention is given to tracing a single idea over the centuries, this idea is important enough for this to be done without risk of exaggeration.

There is, however, a serious flaw in Dr Emerton's book. For reasons of length, and because "it involves a great many biological complexities that distract the mind from the concept of form itself", the whole subject of organic form has been omitted in order to deal exclusively with inorganic crystalline minerals. And so the title of the book promises more than is delivered. The true historical scope of the subject is therefore reduced by focusing on the single strand of inorganic form and the book ends in Abbé Haüy's theory of crystal form, seen as a culmination of Aristotle's search for forms. But this rests on the assumption that from Aristotle up to the eighteenth century there existed a clear division in natural philosophy between things organic and inorganic. Not only is this untrue but crystalline materials were themselves the subject of debate on

this very point. Dr Emerton underestimates the support in the seventeenth and eighteenth centuries for the supposed kinship of crystals and plants. Homberg may have dismissed the chemical "tree of Diana" (a crystalline silver salt of branching form) as an inorganic deposit; but in 1706 Nicole Lemery thought that he could detect hollow tubes, like those in plants, in a tree-like crystalline formation of an iron salt. The botanist Tournefort believed that the crystalline tree of Diana multiplied by a germ similar to the seed of moss or fern. And readers of the article "Crystallization" in the second edition of the *Encyclopaedia Britannica* (1778 – 1783) were given a cross-reference to the article on "Vegetation" where the growth of plants was discussed. This was no slight analogy; the author indicated that crystals, like plants, required an aqueous medium for their generation, and perhaps air as well. He was certain that the tree of Diana was so much like a plant that "a common mode of formation could hardly be denied".

Moreover, at about the same time, de la Méthérie, editor of the *Journal de Physique*, was convinced that all things were products of crystallization: for example that the fetus crystallized from the semen of male and female, and that if animals and plants were rounded instead of polyhedral it was merely due to the impurity of the crystallizing liquids. Even in the early twentieth century the eccentric Haeckel argued that crystals were alive. None of this is discussed by Dr Emerton and her book is poorer for it. If Aristotle's search for forms continued to inspire natural philosophers, so indeed did his conclusion that it was impossible to discover a sharp division between things lifeless and living.

The discussion in the opening chapter of views on the figuration of minerals from antiquity to the seventeenth century is followed by one which gives the philosophical "background" of the forms of Aristotle and his mediaeval commentators. Similarly, chapters on the corpuscular interpretation of form are followed by a "background" chapter on Plato and his followers. This recurring introduction of background information prevents a continuously flowing narrative. The book could have been better written; it reads more like a series of papers than a continuous monograph.

On the other hand, the reappearance of the idea of form in various guises over the centuries is well described and the discussion of what chemists meant by "spirits" and "seeds" is particularly rewarding. With the reservations outlined above, this book will be useful for historians of ideas to consult when they are considering the subject of form. □

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Advanced tunnelling

W.A. Phillips

Principles of Electron Tunneling Spectroscopy. By E.L. Wolf. Clarendon: 1985. Pp.576. £60, \$80.

THE passage of particles through potential barriers, forbidden by classical physics, is convincing evidence in favour of quantum mechanics. Naturally occurring examples of this electron tunnelling are common, but the fabrication of sufficiently thin ($\sim 2\text{nm}$) and perfect artificial barriers is not easy. The first examples of such barriers, apart from tunnel diodes, were aluminium-aluminium oxide-metal junctions (1959). Measurements of current as a function of voltage across the junction not only illustrated the phenomenon of tunnelling but also made an immediate impact when used to explore fundamental aspects of superconductivity. Most of the papers published on tunnelling in the following 15 years were concerned with superconductivity, and were very important in confirming the microscopic theory. Most dramatic of all was the discovery of Josephson tunnelling, where a current can pass from one superconductor to another through an insulating barrier even in the absence of a voltage across the junction, work which culminated in the award of a Nobel prize to Esaki, Giaever and Josephson in 1973.

More recently, the emphasis of research in this area has shifted and broadened. Inelastic tunnelling processes, where electrons lose energy as they tunnel, provide an additional channel for conduction above a threshold bias voltage which is equal to the characteristic energy of, for example, the vibrations of a molecule absorbed in the barrier. This novel spectroscopy, which needs only a voltmeter, is used to study the surface chemistry of absorbed molecules, vibrational states and other excitations in the barrier region. Of particular interest is a related process in which visible light is emitted indirectly by a tunnelling electron in a junction. Technically it is now possible to demonstrate tunnelling through a vacuum gap between a metal tip and a metal surface and so, because of the exponential variation of current with separation, to study surface topography in great detail. The preparation of sufficiently thin films on an atomic scale in the semiconductor industry suggests that interest in tunnelling effects and devices is going to increase sharply.

The appearance of a comprehensive account of electron tunnelling is therefore timely. Wolf has had to make a difficult choice between giving due weight to all aspects of the existing literature, or preparing the ground for future developments by under-playing mature fields of study. He has adopted the former approach, taking an encyclopaedic view

and including 250 figures and 1,250 references, mainly to the original literature. This has meant, inevitably, that roughly half of the book is devoted to aspects of tunnelling that involve superconductivity.

The other problem that Wolf has faced is that while the basic idea of electron tunnelling is relatively straightforward, many of the areas of physics where it can usefully be applied are not. For example, superconductivity, scattering by magnetic impurities or electron scattering by molecules are all complicated topics which have a much broader significance than he has been able to discuss. The consequent unevenness in depth of treatment would be a serious flaw in an introductory book in which complete coverage and continui-

ty are essential, but matters much less in an advanced text such as this: a great deal of background knowledge is assumed and Wolf essentially summarizes the relevant literature on each aspect of the subject and points the reader towards the links between them.

Principles of Electron Tunneling Spectroscopy stands as an important guide to the experimental and theoretical advances of the past 25 years, giving due weight to established knowledge and hinting at developments to come. It will be used extensively by those working in the field. □

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Ways of resistance

Peter I. Trigg

Essential Malariology, 2nd Edn. By Leonard Jan Bruce-Chwatt. Heinemann Medical/Wiley: 1985. Pp.452. Hbk £22.50, \$40. ELBS edition for sale in Africa, Asia and the West Indies, pbk £7.50.

MALARIA is still the most important parasitic disease in the tropics. Excluding Africa south of the Sahara, 5.5 million cases were provisionally reported to the World Health Organization (WHO) in 1983 but the true incidence is estimated to be around 20 million cases per year. A further 200 million people in Africa are believed to be chronically infected, one-third of whom suffer acute manifestations.

This overall epidemiological picture has remained unchanged since the publication of the first edition of *Essential Malariology* in 1980. However, the technical constraints on malaria control, amply described by Professor Bruce-Chwatt, have increased. Probably the most important of them has been the spread of multi-resistant *Plasmodium falciparum* in South-East Asia and Oceania. Chloroquine resistance has appeared in Africa and by 1984 had been reported in 15 countries of Central and East Africa. This situation has provoked a renaissance in malaria research, with, over the past five years, remarkable progress in the development and field testing of new antimalarial drugs, the development of prospective vaccines and the understanding of the epidemiology of the disease and its socioeconomic impact.

Against this backdrop, the new edition of *Essential Malariology* comes at an opportune time. Although it is a pity that it appeared too late to include all of WHO's recent recommendations on the use of antimalarial drugs — an understandable omission in such a rapidly moving field — it certainly provides, at a critical juncture in malaria control, the scientific background to recent parasitological,

entomological, epidemiological and immunological research on human malaria and its transmission. In doing so it goes beyond the scope of the first edition. Professor Bruce-Chwatt faced a difficult problem in selecting practical information from the avalanche of recent publications, but he has admirably outlined important areas and has highlighted the relationship between basic research and its application.

The new edition also stresses practical methods of prevention, diagnosis, treatment and control in the context of the current policy of integrating malaria control into an overall primary health care approach. Various patterns of such integration have been developed in different countries, depending on epidemiological, administrative and socioeconomic conditions and systems of government. The success of these programmes depends on suitable education of medical and health personnel, and a flexible approach in guiding antimalarial operations. A major effort to train such personnel is being made by international and national organizations, as an essential prerequisite to the translation of advances in malaria research into useful measures in the field. This clearly and simply written book will without doubt play an important role in such training efforts. □

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New editions

- *The Birth of A New Physics: Revised and Updated*, by I. Bernard Cohen. Publisher is W.W. Norton, price is \$17.95. To be published in Britain in October, £15.95.
- *Amino Acid Metabolism*, 2nd Edn, by David A. Bender. Publisher is Wiley, price is \$38, £24.
- *Introduction to Mass Spectrometry*, 2nd Edn, by J. Throck Watson. Publisher is Raven, price is \$49.
- *Hematology*, 4th Edn, edited by William S. Beck. Publisher is MIT Press, price is hbk \$40, £39.95; pbk \$18.50, £18.50.

Digging into Egypt

Glyn Daniel

Flinders Petrie: A Life in Archaeology. By Margaret S. Drower. *Gollancz/David & Charles*: 1985. Pp. 500. £20, \$55.

It is 43 years since Sir Flinders Petrie died in a hospital in Jerusalem, and a full biography and critical assessment of his work has long been overdue. He was without any question one of the heroic figures of British archaeology, that *galère* which includes Layard, Rawlinson, John Evans, Arthur Evans, Pitt-Rivers, Woolley and Mortimer Wheeler — men who revolutionized our understanding of mankind's ancient past.

Petrie's autobiography, *Seventy Years in Archaeology*, appeared in 1931 (born in 1853, he reckoned that his archaeological career started at the age of eight, when he began collecting coins) and a sympathetic and colourful picture of him was given by Margaret Murray in *My First Hundred Years* (1963). Ma Murray was devoted to "Proffy": it was commonly believed by his students and hers that she had in her youth aspired to marry Petrie. She had worked as his assistant from 1892 onwards, and remained on formal terms with Lady Petrie: after the 1902 season at Abydos she had never again been invited to join one of the Petrie expeditions to Egypt or Palestine. Indomitable, indispensable, five-foot high, she held the fort in University College London while Petrie worked in the field.

Margaret Drower (Mrs Hackforth Jones) was one of the last three students to take Petrie's Diploma Course in Egyptology. In the summer of 1929 she went to see the great man and later wrote, "I have a memory of twinkling eyes, an unexpectedly high, light voice, bushy eyebrows and a patriarchal white beard". She tells us that Petrie took aspirant students to lunch at his favourite teashop, the Express Dairy, at the corner of Gower Street: if the candidate "chose a frugal meal, he met with approbation, if he plumped for meat and two vegetables with a sweet to follow, he scored zero and was unlikely to be accepted".

This is a very long book, well over a quarter of a million words — but it is authoritative, comprehensive and fully documented: it includes lists of all Petrie's excavations and publications, and maps of all the sites mentioned. Everyone concerned with the history of Egyptology and with Petrie, who was the father of British Egyptology, will have to make this fine biography their starting point and main

reference. It is first and foremost the chronicle of a man and his life's work and there is no starry-eyed idolatry. His difficulties and quarrels and mistakes are listed as well as his triumphs and achievements. We are reminded that while Petrie introduced new methods into Egyptian excavation, there is no mention of stratified records in his *Methods and Aims in Archaeology*.

Throughout his life Petrie was at loggerheads with Grafton Elliot Smith, who had been Professor of Anatomy in the Cairo School of Medicine but had never visited any of Petrie's excavations. They clashed over the nature and techniques of Egyptian mummification, but most of all over Elliot Smith's extravagant theories of the

card, the stick as a marker, and the card, when carefully sighted along two edges, to give him an accurate right-angle. It has always seemed to me incredible that the author of *Inductive Metrology*, and the man who surveyed Stonehenge, the hill-figures of England and the Pyramids of Egypt so accurately could have been working in Wales with a peastick and a visiting card. In the book, Wheeler's tale is cut down to size: "In fact he would almost certainly have had with him his long surveying tape, and probably a small theodolite as well — such tales grow in the telling".

Margaret Drower is very good and fair in dealing with Petrie's parerga — Stonehenge, Silbury Hill, hill-figures, Geoffrey of Monmouth, the laws of Hammurabi, mediaeval Wales and many another subject which occupied his restless, polymathic mind. But it is as the great pioneer Egyptologist that he will be remembered. How does she see him? Some have said he was no scholar, just an excavator: others like Allbright described him as "the greatest archaeological genius of modern times". Margaret Drower is scrupulously honest and gives us an admirably balanced portrait of this quite remarkable man who worked with amazing energy and perseverance until the end of his life, and was exemplary in publishing promptly the results of his work. What was the driving force? Not, I think, the overall revelation of the life and manner of the ancient Egyptians and the role of ancient Egypt in the prehistoric and protohistoric world.

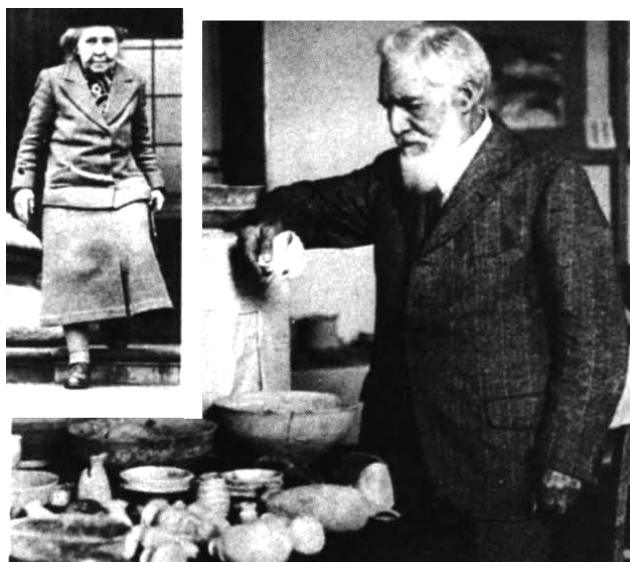
While he was an obsessional excavator and collector, few of his excavations were project orientated.

When Petrie was admitted to hospital in Jerusalem he expressed the desire that if he died his head should be given to the Royal College of Surgeons in London "as a specimen of a typical British skull" and that his brain should be preserved for scientific investigation. This was done: his head was removed the night he died. It did not arrive in London until the end of the war, and for a while it was lost until an astonished assistant moving away some piles of books came face to face with Sir Flinders Petrie who had been dead for ten years. It was a macabre situation which would have amused the old man who wrote:

Let there be no regrets, now that this life is past
For earth, and friends, and work have all been
sweet to me

And labour garnered safe has laid the ages clear
That all may plainly see what none had thought
before.

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Petrie at the opening of his first Palestinian exhibition, London, July 1930. And (inset) Margaret Murray: "Indomitable, indispensable, five-foot high, she held the fort in University College while Petrie worked in the field".

Egyptian origins of all things which he set out in his *The Ancient Egyptians and the Origins of Civilization* (1915). Petrie was present at a meeting of the British Association in Manchester in 1915 when Elliot Smith and his disciple W. J. Perry spoke of the Egyptians evolving an "Archaic Heliolithic Civilization" which spread around the world. Petrie wrote in his diary "much disgusted", and his copy of Elliot Smith's book is full of pencilled comments: "No such thing", "Nonsense!", "No, No!", "No evidence whatsoever". The two men were colleagues at University College London for many years and they were hardly on speaking terms. If Margaret Murray is to be believed, they made it up at last, before they both retired: but I doubt this.

There are many Petrie myths. Margaret Drower scotches one which I had never believed. Mortimer Wheeler was a friend and admirer of Petrie and often entertained him on his Welsh excavations. In *Still Digging*, Wheeler describes how Petrie, then 73, made surveys of sites in the Brecon Beacons, his only equipment being a bamboo peastick and a visiting

Chemical mechanisms of acid generation in the troposphere

Jack G. Calvert, Allan Lazrus, Gregory L. Kok, Brian G. Heikes, James G. Walega, John Lind & Christopher A. Cantrell

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Diverse chemical pathways in the troposphere convert sulphur and nitrogen oxides and organic compounds into acids, involving the gas phase, the liquid phase (cloud, fog and rain water) and, possibly, certain suspended aerosols. The rates of acid generation are critically affected by the extent of generation of the oxidizing species and the kinetics of the reactions. Precipitation in the eastern United States shows a strong seasonal variation in deposition of sulphates in contrast to nitrates. Computer simulations can now rationalize the observed seasonal trends. Recent tropospheric measurements of gaseous hydrogen peroxide show that this gas is a major oxidant leading to sulphuric acid generation in cloud water.

CONCERN has grown over the biological impact of acid deposition on ecologically sensitive regions in northern Europe and eastern North America. The extent of the potential problems induced by acid deposition and the timescales for damage and recovery of biological systems remain ill defined¹⁻¹⁴. The evidence is clear that, in lakes that have been acidified by such deposition, the detrimental effects on the fish population can be dramatic¹⁴. It is difficult to infer from existing published information to what extent acid deposition contributes to the increasing levels of forest damage which have been observed in New England (United States), the Black Forest (West Germany) and elsewhere in recent years. Although this damage was originally attributed to acid deposition alone, exposure to oxidants (such as ozone and peroxyacetylnitrate), metal ions, insects and periods of drought may also play a part. However, there is general agreement that in areas in which there is a poor

buffering capacity and in which ecologically sensitive organisms exist, acid deposition is a potentially serious problem, and may be a present danger.

Trends in acid deposition in eastern North America are difficult to establish from the limited database available. However, some interesting observations bearing on the mechanism of acid generation can be made from MAPS3S/RAINE data which were collected at eight stations in the eastern United States during 1977-79 (ref. 15). Data from four of the stations (Whiteface, New York; Ithaca, New York; Pennsylvania State University; Charlottesville, Virginia) that lie near a line to the north-east (latitude, 38-44° N), provide reasonably complete data sets for the entire 3-year period. Data for sulphate and nitrate ion deposition (analogues for sulphuric and nitric acids, respectively) and total amounts of precipitation are shown in Fig. 1a, b and c, respectively. A seasonal variation in the sulphate

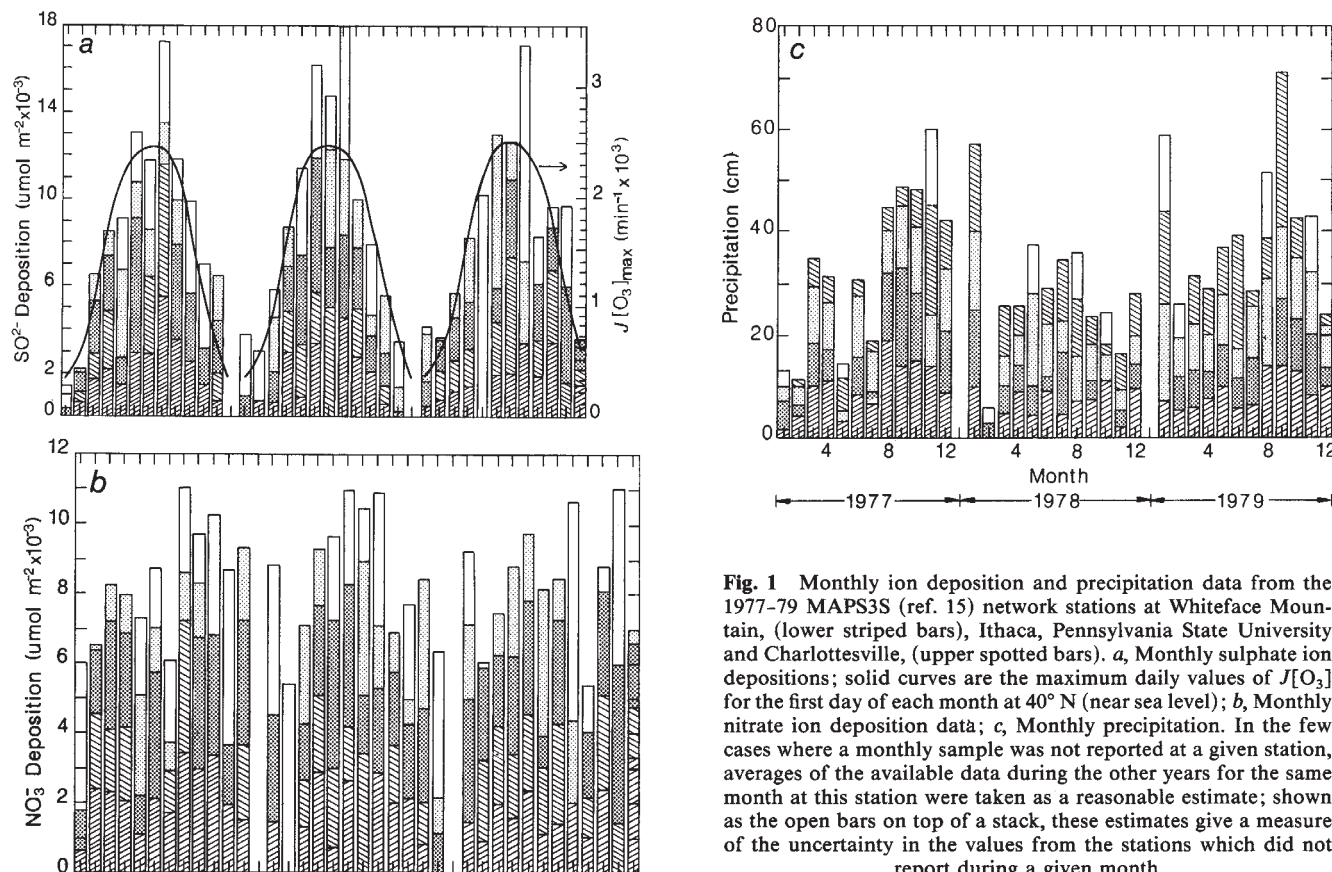


Fig. 1 Monthly ion deposition and precipitation data from the 1977-79 MAPS3S (ref. 15) network stations at Whiteface Mountain, (lower striped bars), Ithaca, Pennsylvania State University and Charlottesville, (upper spotted bars). *a*, Monthly sulphate ion depositions; solid curves are the maximum daily values of $J[\text{O}_3]$ for the first day of each month at 40° N (near sea level); *b*, Monthly nitrate ion deposition data; *c*, Monthly precipitation. In the few cases where a monthly sample was not reported at a given station, averages of the available data during the other years for the same month at this station were taken as a reasonable estimate; shown as the open bars on top of a stack, these estimates give a measure of the uncertainty in the values from the stations which did not report during a given month.

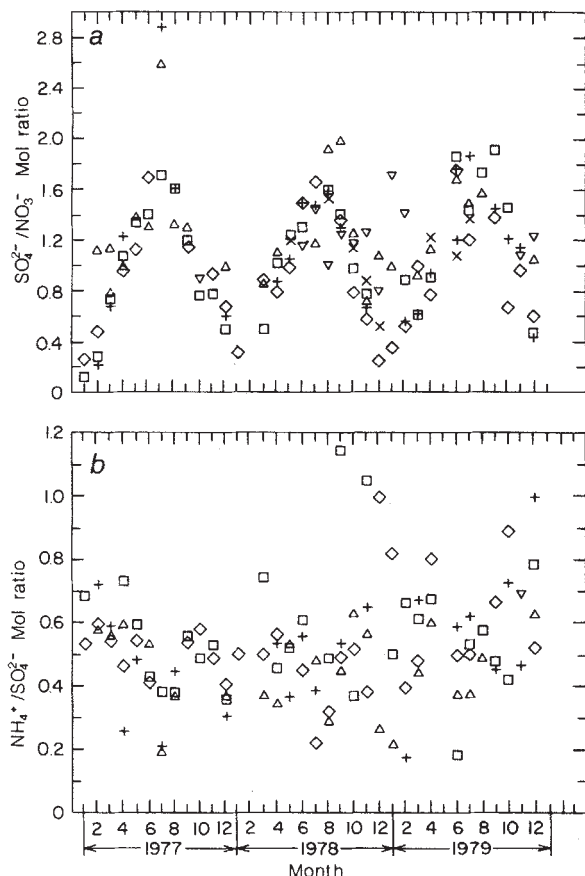


Fig. 2 Ratios of the ion concentrations from the deposition data from several of the 1977–79 MAPS3S (ref. 15) network stations. □, Whiteface Mountain; +, Ithaca; ◇, Pennsylvania State University; △, Charlottesville; ×, Urbana, Illinois; ∇, Brookhaven. *a*, Monthly sulphate ion/nitrate ion molar ratios; *b*, Monthly ammonium ion/sulphate ion molar ratios.

deposition is apparent in Fig. 1*a*, with highest values in the summer months and lowest in the winter. A similar seasonal effect has been noted for SO_4^{2-} deposition by Raynor and Hayes¹⁶ from samples collected at Brookhaven National Laboratory and by Rodhe and Granat¹⁷ from estimates of SO_2 oxidation rates over Europe. There is no obvious seasonal correlation for nitrate deposition (Fig. 1*b*). Wet deposition of sulphate and nitrate species is not observed unless there is some form of precipitation to deliver them to the ground. Yet there is no apparent correlation between the amount of precipitation and the sulphate or nitrate deposited during a particular month. For example, although the highest precipitations occurred in September 1979 and January 1978 and 1979, sulphate and nitrate depositions were not unusually high during these months. A definite seasonal variation can be seen in the $\text{SO}_4^{2-}/\text{NO}_3^-$ molar ratio at each station (Fig. 2*a*), while the $\text{NH}_4^+/\text{SO}_4^{2-}$ molar ratios show no obvious seasonal variations (Fig. 2*b*). We will use this set of data to test our hypotheses concerning the mechanism of acid generation and deposition in the eastern United States.

Much of the fundamental chemistry and physics relating to the acid deposition problem are reasonably well established. Yet in the development of sophisticated transport and transformation models, it is extremely complex to treat accurately the many processes that lead to acid deposition: (1) the amounts and locations of the emission of both anthropogenic and natural sulphur, nitrogen and organic compounds; (2) the rates of chemical transformation of sulphur and nitrogen oxides and organic compounds to acids; (3) the transport and dispersion of the acids and their precursors over the hundreds of kilometres which may separate large source and sensitive receptor areas; and (4) the rates of dry and wet deposition of the acids and

their precursors. In this discussion we attempt to evaluate our present knowledge of one important aspect of this problem: the chemical mechanisms leading to acid production in the troposphere. The detailed mechanisms of acid generation in the troposphere are of more than academic interest in that transport and transformation models are sensitive to the choice of transformation rates used¹⁸. This sensitivity results largely from the differences in the ease with which the acid precursors and the acids themselves are removed from the atmosphere. Once SO_2 is oxidized to sulphuric acid aerosol, it is dry deposited less rapidly than is SO_2 . If aerosols composed of sulphuric acid and its salts (such as ammonium bisulphate and ammonium sulphate) are incorporated into precipitating clouds, then the deposition of these species can be faster than that of gaseous SO_2 . On the other hand, the nitric acid developed in the troposphere is much more rapidly deposited on the surface of the Earth than are its precursors, NO and NO_2 . Thus, the amounts and the chemical nature of the acids deposited at sites many kilometres from the source of the precursors are sensitive functions of the rates with which the acids are developed from the precursors.

There have been many recent reviews of the chemistry of acid development in the troposphere, and a consensus on the important reactions leading to acid generation is rapidly developing^{19–26}.

Gas-phase processes

Both gas- and liquid-phase chemical processes seem to be important sources of acid generation within the troposphere. Gas-phase reactions not only generate sulphuric and nitric acids through the oxidation of SO_2 and the oxides of nitrogen (NO , NO_2), but they also lead to the generation of several oxidants that are important reactants for acid generation in the liquid (cloud) phase.

As a guide to our considerations of the chemistry of acid development, we consider the results of computer simulations of the gas-phase chemistry expected to occur at the extremes of the seasons, 1 January and 1 July at 40° N latitude. The reaction scheme used is a new version of that described by Stockwell and Calvert²⁷ in which the NO_x chemistry has been updated in view of recent studies²⁸, and the zenith angle-dependent photochemical processes are treated in a more realistic fashion. The chemistry was simulated in the chemical model (no transport included) using four different scenarios of initial concentrations and impurity emissions which we believe to be representative of the non-urban atmospheres of eastern United States during these seasons (see Table 1). The rate constants of the chemical reactions were varied with chosen temperature profiles. The ranges of the temperatures used, 16 to 30 °C for 1 July and –6 to 4 °C for 1 January, are representative of those encountered during these days at stations in the eastern United States near 40° N latitude. *J* values (the apparent first-order rate constants for molecules undergoing photodecomposition) for the 16 photochemical processes included in the mechanism were calculated for elevations near sea level and in accord with the seasonal and diurnal variations in the solar irradiance and the known absorption and quantum yield data for these species. Here $J(x) = \int (\sigma)_x (\phi_x)_\lambda (I)_\lambda d\lambda$, where $(\sigma)_x$, $(\phi_x)_\lambda$ and $(I)_\lambda$ represent the absorption cross-section of the *x*-species, the primary quantum yield of the particular process involving *x* and the solar irradiance for the small wavelength interval from λ to $\lambda + d\lambda$, respectively; the integral is over all wavelengths for which the $\sigma\phi I$ product is non-zero. The large difference between the July and January *J* values for NO_2 and O_3 is shown in Fig. 3. In theory these differences play a large role in defining the seasonal variations observed in acid generation in the troposphere.

SO_2 , NO_2 and hydrocarbons are oxidized to acids by several oxidizing agents which are formed in the gas-phase reactions within the troposphere. The important reactive species include molecules such as ozone, hydrogen peroxide, methyl hydroperoxide and peroxyacetic acid, and reactive free radicals such as HO, NO_3 and HO_2 . The mechanisms of their formation, involv-

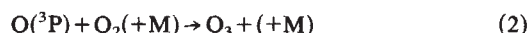
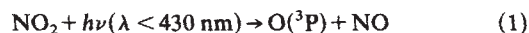
Table 1 Initial conditions for simulations of acid generation in the troposphere

Case	[H ₂ O]	[O ₃]	[NO]	[NO ₂]	[SO ₂]	Concentration (p.p.b.)		[CO]	[CH ₂ O]	[Ald]	[Ket]
						[RH-1]	[RH-2]				
Midsummer (1 July)											
I	3.1 × 10 ⁷	30	7.5	2.5	6.0	12.5	50	200	2	1	0.5
II	3.1 × 10 ⁷	20	0.75	0.25	0.6	1.25	5	100	0.2	0.1	0.05
Midwinter (1 January)											
III	3.6 × 10 ⁶	30	7.5	2.5	6.8	21.1	84	200	2	1	0.5
IV	3.6 × 10 ⁶	20	0.75	0.25	0.68	2.11	8.4	100	0.2	0.1	0.05

[CH₄] was fixed at 1,400 parts per 10⁹ (p.p.b.) in each run. RH-1 and RH-2 are alkene and alkane hydrocarbons; Ald and Ket represent the higher aldehydes and ketones, respectively. Net emission-deposition rates of insertion of NO, NO₂ and the hydrocarbons were adjusted so that there was little change in their concentrations after simulation times of 24 h.

ing NO, NO₂, hydrocarbons, aldehydes and sunlight, are now reasonably well characterized¹⁹⁻²⁶.

The concentration of ozone is established in the troposphere through the photolysis of NO₂ (reaction (1)) followed by reaction (2), and to an undefined extent, by the insertion of stratospheric ozone through stratospheric-tropospheric folding events.



It is removed in part by reaction (3) with NO to re-form NO₂:



The cycle of reactions (1)–(3) which occurs during the day leads to a concentration of ozone within the troposphere which is given approximately (usually within a factor of 2)²⁹ by relation (4):

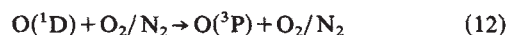
$$[\text{O}_3] \approx [\text{NO}_2]J(1)/[\text{NO}]k_3 \quad (4)$$

The apparent first-order photodissociation constant for reaction (1), $J(1)$, is proportional to the sunlight intensity present in the troposphere in the wavelength range which causes the dissociation of NO₂ (290–430 nm). Thus, the ozone concentration usually grows as the sunlight intensity increases during the day, and the [NO₂]/[NO] ratio climbs as NO is converted to NO₂. The observed NO to NO₂ conversion is promoted through chain reactions (5)–(9) involving the HO, HO₂ and organic peroxy radicals (RO₂) formed from the hydrocarbons and their oxidation products (such as aldehydes and carbon monoxide).



R'COR'' represents a ketone or aldehyde; R' and R'' are H-atoms or organic radicals such as CH₃ and C₂H₅.

The important hydroxy radical (HO) which initiates this sequence of reactions is formed in the relatively clean atmosphere largely through the photodecomposition of ozone:



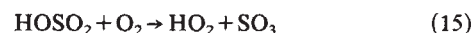
Thus, the HO radical concentration that develops in a tropospheric air mass is a complex function of the concentration and the nature of the hydrocarbons present, [NO], [NO₂], humidity and the intensity of the solar radiation at wavelengths <306 nm which control the magnitude of $J(\text{O}_3)$, the apparent first-order rate constant for reaction (10). Note in Fig. 3 (dashed curves) that $J(\text{O}_3)$ values for winter are much lower than those of summer (40° N latitude). Two major factors result in the lower rates of oxidant generation in the winter than in summer: there

is a lower rate of O(¹D) generation in reaction (10) in winter, and the fraction of O(¹D)-atoms that generate HO radicals in reaction (11) decreases at the lower [H₂O] characteristic of the winter months, as the unproductive reaction (12) gains importance.

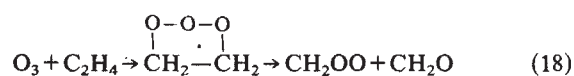
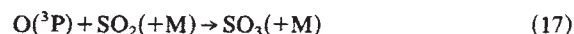
The reactions of the HO free radical with SO₂ and NO₂ are judged to be the major, gas-phase sources of sulphuric and nitric acids, respectively:



The HOSO₂ radical product of reaction (13) leads ultimately to sulphuric acid. The relative importance of the alternative pathways for the reactions of this free radical has been studied previously^{19,30-35}. The most recent work suggests strongly that reaction (15), a chain-carrying step, represents its dominant fate in the atmosphere^{34,35}; the SO₃ product of reaction (15) reacts rapidly to generate H₂SO₄ in reaction (16):



Other gas-phase mechanisms of SO₂ oxidation are expected theoretically; reaction (17) can occur with O(³P) atoms and reaction (19) with Criegee intermediates (for example, CH₂OO) which are formed from ozone-alkene reactions such as reaction (18):



Theory suggests that oxidation of SO₂ in reaction (17) can be important only in stack NO₂- and SO₂-rich gas plumes,

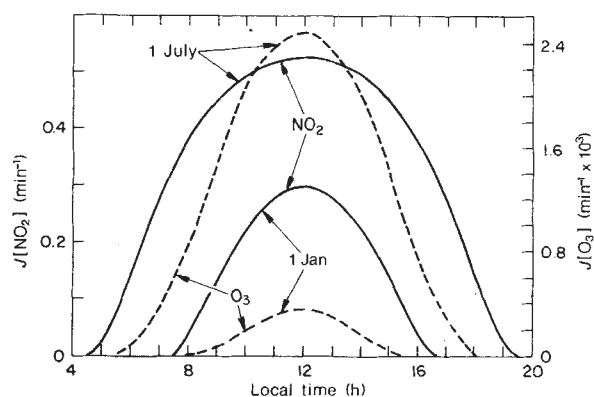
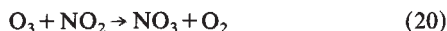


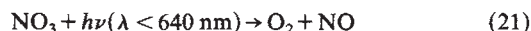
Fig. 3 Theoretical diurnal variation of the $J[\text{NO}_2]$ and $J[\text{O}_3]$ values for midsummer and midwinter at 40° N latitude near sea level.

whereas reaction (19) may be significant only in highly polluted urban atmospheres. The dominance of the HO-SO₂ reaction in the gas-phase generation of H₂SO₄ has been suggested in previous studies involving computer simulations²⁴⁻²⁶. For air masses with concentrations of impurities in the range common to the eastern United States, reaction (13) usually provides >98% of the H₂SO₄ generated in gas-phase reactions. It is instructive to compare the total rates of SO₂ oxidation from gas-phase reactions as estimated by computer simulation (Fig. 4). They exhibit both seasonal and diurnal variations which reflect largely the changing rates of generation of HO radicals in the photochemical processes involving O₃, reactions (10)–(12), and other species. In midsummer under clear skies the maximum rate is ~9% h⁻¹ for the conditions of case I and somewhat lower (6% h⁻¹) for the cleaner atmosphere of case II. Our previous simulations have shown that the rates of SO₂ oxidation expected for the highly polluted and very clean atmospheres are both somewhat lower than those for the air masses of intermediate levels of impurities such as we have considered here²⁴. These midsummer rates are not insignificant when one considers that theoretically they will result in 34–49% conversion of an initial quantity of SO₂ to acid during a 24-h clear period. Simulations for the midwinter cases III and IV show much lower rates, with only 3.1–4.7% conversion of SO₂ in a 24-h period.

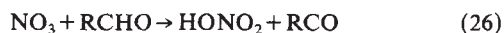
Current reaction rate data and computer simulations of tropospheric chemistry suggest that the gas-phase oxidation of NO₂ proceeds much more rapidly than that of SO₂, largely through reaction (14) during daylight. The rate constant for this HO radical reaction is ~10 times that for reaction (13) with SO₂. During the night there is an additional reaction sequence which is expected to lead to nitric acid as well; it is initiated by the ozone molecule reactions with NO₂ (refs 19, 24–28).



The NO₃ radical product of reaction (20) is a relatively unimportant source of HONO₂ during the daylight hours, as it is destroyed readily through its rapid photolysis in sunlight and by its rapid reaction with NO:



The generation of NO through NO₂ photolysis in reaction (1) and NO₃ loss in reactions (21) and (22) cease at night, and much of the existing NO is oxidized to NO₂ by ozone after sunset. Thus, the loss of NO₃ in reactions (21)–(23) becomes unimportant and the NO₃ concentration rises at sunset (see Fig. 5a for simulations). Various acid-forming reactions of NO₃ can become important during the night. For example, NO₃ reacts with NO₂ to form N₂O₅ in reaction (24) (Fig. 5b), and this may react with water to form nitric acid in reaction (25) or it may form the acid directly by H-atom abstraction from aldehydes and other reactive compounds [reaction (26)]:



The rate constant for the gas-phase reaction (25) between N₂O₅ and H₂O is uncertain, as undetected heterogeneous processes can lead to an overestimate of this quantity. Recent tropospheric measurements of Platt *et al.*³⁶ give $k_{25} < (2.9 \pm 0.9) \times 10^{-21} \text{ cm}^3 \text{ per molecule per s}$. Other recent laboratory estimates of Tuazon *et al.*³⁷ give $k_{25} < (1.3 \pm 0.2) \times 10^{-21} \text{ cm}^3 \text{ per molecule per s}$. As the accuracy in k_{25} estimates has improved, it has tended towards zero; we have assumed $k_{25} = 0$ in the present simulations. Figure 4b shows that the gas-phase chemistry alone can, in theory, convert NO₂ to HNO₃ at very significant rates. For midsummer cases I and II, ~18 and 26% h⁻¹, respectively, of the NO₂ can give nitric acid in gas-phase reactions; in midwinter, rates are much lower in theory,

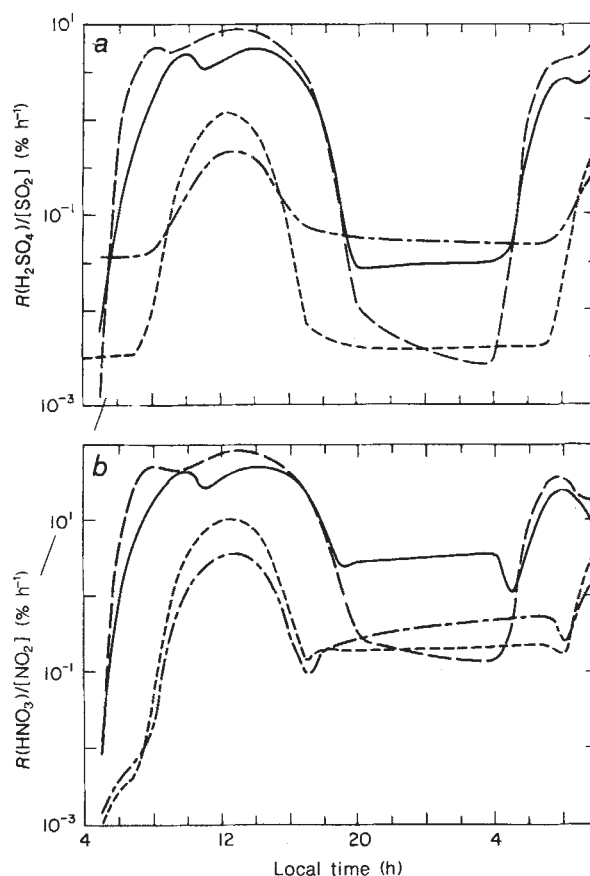


Fig. 4 Theoretical rates of SO₂ (a) and NO₂ (b) conversion to acids for midsummer and midwinter scenarios I–IV of Table 1. Solid lines, summer I; long dashed lines, summer II; long-short dashed lines, winter III; short dashed lines, winter IV.

0.8 and 1.9% h⁻¹ for cases III and IV, respectively. Thus, if gas-phase chemistry alone were operative during a 24-h period, 98.5 and 99.8% of an initial quantity of NO₂ can be oxidized to nitric acid for midsummer cases I and II, respectively, and about 18 and 37% for the midwinter cases III and IV, respectively. If the recent values of Tuazon *et al.*³⁷ are correct, the 24-h average rates of NO₂ gas phase oxidation are significantly higher than these estimates. Solution-phase reactions can also contribute, especially to the winter period rates as discussed below.

Gas-phase reactions can also generate organic acids by various mechanisms²⁴. Thus, reactions of the Criegee intermediates with, for example, H₂O vapour or HO₂ radicals with aldehydes, can lead to formic acid, acetic acid and the higher organic acids. The theoretical fraction of total acid that is composed of organic acids is relatively large; for tropospheric air masses with the typical range of compositions of the impurity gases present, one expects in theory between 5 and 20% of the total acids to be organic acids²⁴.

Aqueous-phase cloud-water chemistry

Sulphuric acid generation. Many of the good oxidizing agents formed in the gas-phase chemistry in the troposphere such as ozone, H₂O₂, peroxyacetyl nitrate, methylhydroperoxide and peroxyacetic acid, do not react with gaseous SO₂ at measurable rates, although the thermodynamic potential for reaction is great. When these species are dissolved in cloud water they readily oxidize dissolved SO₂ (largely in the form of HSO₃⁻ ions).

Current estimates of the relative significance of the various possible tropospheric oxidants for bisulphite in cloud water can be compared in Fig. 6. This treatment is similar to that originally given by Penkett *et al.*³⁸, and in subsequent evaluations by Martin²⁰, Schwartz²² and Calvert *et al.*^{24,25}. Compared here are the instantaneous rates of the oxidation of bisulphite ion as

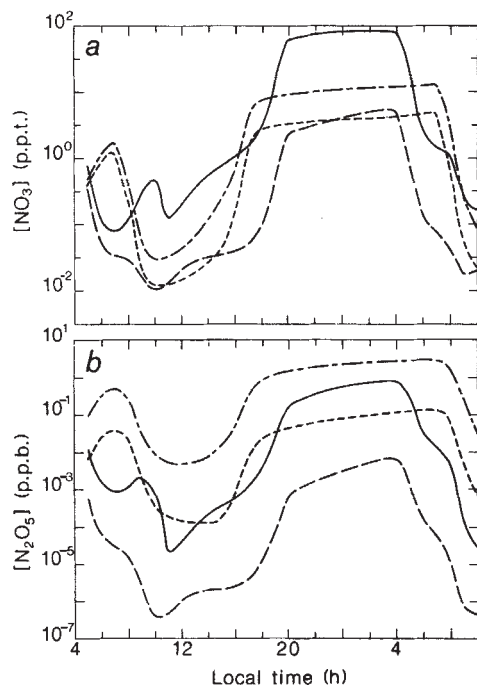


Fig. 5 Theoretical concentrations of NO_3 (a) (in parts per 10^{12} (trillion) p.p.t.) and N_2O_5 (b) as a function of time for the midsummer and midwinter scenarios I-IV of Table 1. Lines are as outlined in Fig. 4 legend.

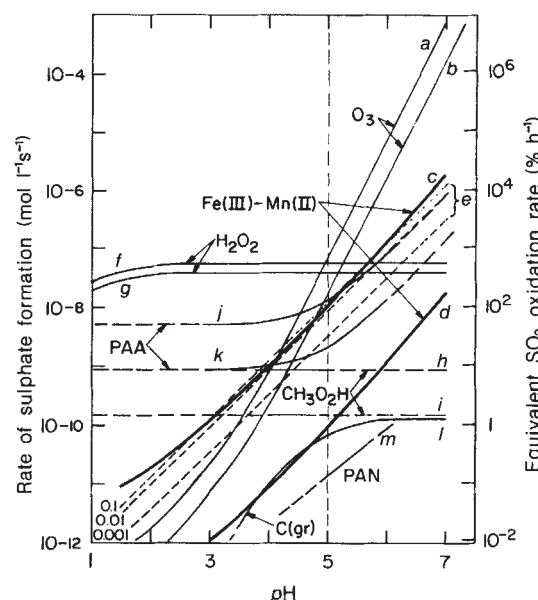


Fig. 6 Theoretical instantaneous rates of liquid-phase oxidation of S(IV) ; $\text{SO}_2(\text{g})$ at 1 p.p.b. in equilibrium with cloud water; left-hand ordinate gives rates independent of the liquid water content (L) of the cloud; right-hand ordinate gives the equivalent gas-phase oxidation rate for SO_2 for a cloud with $L = 1 \text{ g m}^{-3}$; see text for the specific reactant conditions chosen for the calculations in curves a-m.

estimated for typical impurity levels in a non-urban parcel of tropospheric air over eastern North America. We have assumed that the gaseous SO_2 concentration is 1 p.p.b (parts per 10^9), and that this is in equilibrium with cloud water. The left-hand ordinate of Fig. 6 shows the aqueous sulphuric acid production rate in moles $\text{l}^{-1} \text{s}^{-1}$, independent of the liquid water content of the cloud. The right-hand ordinate gives the equivalent gas-phase SO_2 conversion rates for a typical rain cloud with a liquid water content of 1 g m^{-3} of air. The abscissa of the figure is the pH of the cloud water solution. Pure water equilibrated with atmospheric CO_2 alone would have a pH of ~ 5.6 . Charlson and Rodhe³⁹ estimated that the pH of rain water uncontaminated by anthropogenic sources would range from 4.5 to 5.6 because of the variability of the sulphur cycle alone; in the absence of basic materials such as NH_3 , CaCO_3 , average pH values of ~ 5 are expected to occur in pristine locations. Hence the region to the left of the vertical dashed line at $\text{pH} = 5$ is that of common interest in atmospheric considerations of acid rain development. For most of the oxidants considered here, the rates are strongly pH dependent; for all but the reactants H_2O_2 , $\text{CH}_3\text{O}_2\text{H}$ and $\text{CH}_3\text{COO}_2\text{H}$, the rates fall off sharply as the cloud water becomes more acidic; this results largely from the decreased solubility of SO_2 with pH decrease.

For the typical conditions picked for Fig. 6, the highest rates of HSO_3^- oxidation are observed for the reactant ozone in solutions at $\text{pH} > 5$; in these calculations we have assumed that the cloud water is in equilibrium with gaseous ozone at 50 p.p.b. and have accepted the rate constants recommended by Hoffmann⁴⁰. The rates are somewhat slower for 15°C (Fig. 6, curve b) than for supercooled, liquid cloud water at -5°C (curve a). In this case the enhancement of the rate at the lower temperature arises from the increased Henry's law constant (increased solubility) for the reactants which more than offsets the lower rate constant at the lower temperature. In the case of the ozone oxidant, the rate decreases as the cloud water becomes more acidic; at -5°C , rates of 10^4 , 10^3 , 10^2 , 10 and $1\% \text{ h}^{-1}$ are expected for pH values of ~ 5.6 , 5.1 , 4.6 , 4.1 and 3.5 , respectively.

For the lower pH values usually encountered in clouds and rainwater, the theoretical rates of oxidation of S(IV) by H_2O_2 (curves f (-5°C) and g (15°C) of Fig. 6) are also very high

and, more important, the rates are relatively insensitive to the pH over the entire range of values. With H_2O_2 , as well as $\text{CH}_3\text{O}_2\text{H}$ and $\text{CH}_3\text{COO}_2\text{H}$, the decrease in rate which is expected to occur with increased acidity because of the lower SO_2 solubility, is almost completely offset by the increase in rate caused by acid catalysis of the reaction. We have used the evaluation of Hoffmann⁴⁰ in deriving the theoretical rates in this case, and the gas-phase concentration of H_2O_2 which is allowed to equilibrate with the cloud water is 1 p.p.b.; this leads to a $[\text{H}_2\text{O}_2]$ in the cloud water of about $3.4 \times 10^{-5} \text{ M}$ for 15°C scenario, a value representative of the ambient measurements in cloud and rain water in the non-urban atmosphere during the non-winter seasons⁴¹⁻⁴⁵.

In theory, other peroxy compounds such as peroxyacetic acid and methylhydroperoxide can lead to S(IV) oxidation in tropospheric cloud water. These and other organic peroxides, expected from the computer simulations, have not yet been identified in the troposphere. The Henry's law constants and rate constants for $\text{CH}_3\text{O}_2\text{H}$ and $\text{CH}_3\text{COO}_2\text{H}$ reactions with S(IV) have been studied recently by Lind *et al.*⁴⁶. From these data we have estimated the rates of S(IV) oxidation by these oxidants; we have assumed that 1 p.p.b. (total gas and liquid phase) of each oxidant is present (see Fig. 6, curves h (-5°C) and i (15°C) for $\text{CH}_3\text{O}_2\text{H}$ and curves j (-5°C) and k (15°C) for $\text{CH}_3\text{COO}_2\text{H}$). The rates of oxidation of bisulphite ion by these species in cloud water at $\text{pHs} < 5$ are significantly lower than those for H_2O_2 at equivalent total gas- and liquid-phase concentrations. Although the rate constants for these oxidants with S(IV) are nearly equal to those for H_2O_2 , their lower solubilities in water lead to the lower rates.

In theory a highly significant contribution to HSO_3^- oxidation can arise from HO and HO_2 radicals formed in gas-phase reactions but transported to the cloud droplets as suggested by Chameides and Davis⁴⁷ (Fig. 6, curves e). The results shown are for an assumed cloud droplet size of radius $12 \mu\text{m}$ and three different choices for the sticking coefficient of the free radicals on the droplet (α), 10^{-1} , 10^{-2} and 10^{-3} ; α is the fraction of the total collisions between the gaseous species and the surface which result in the capture of the species by the droplet. We have adopted the parameters used by Chameides and Davis for

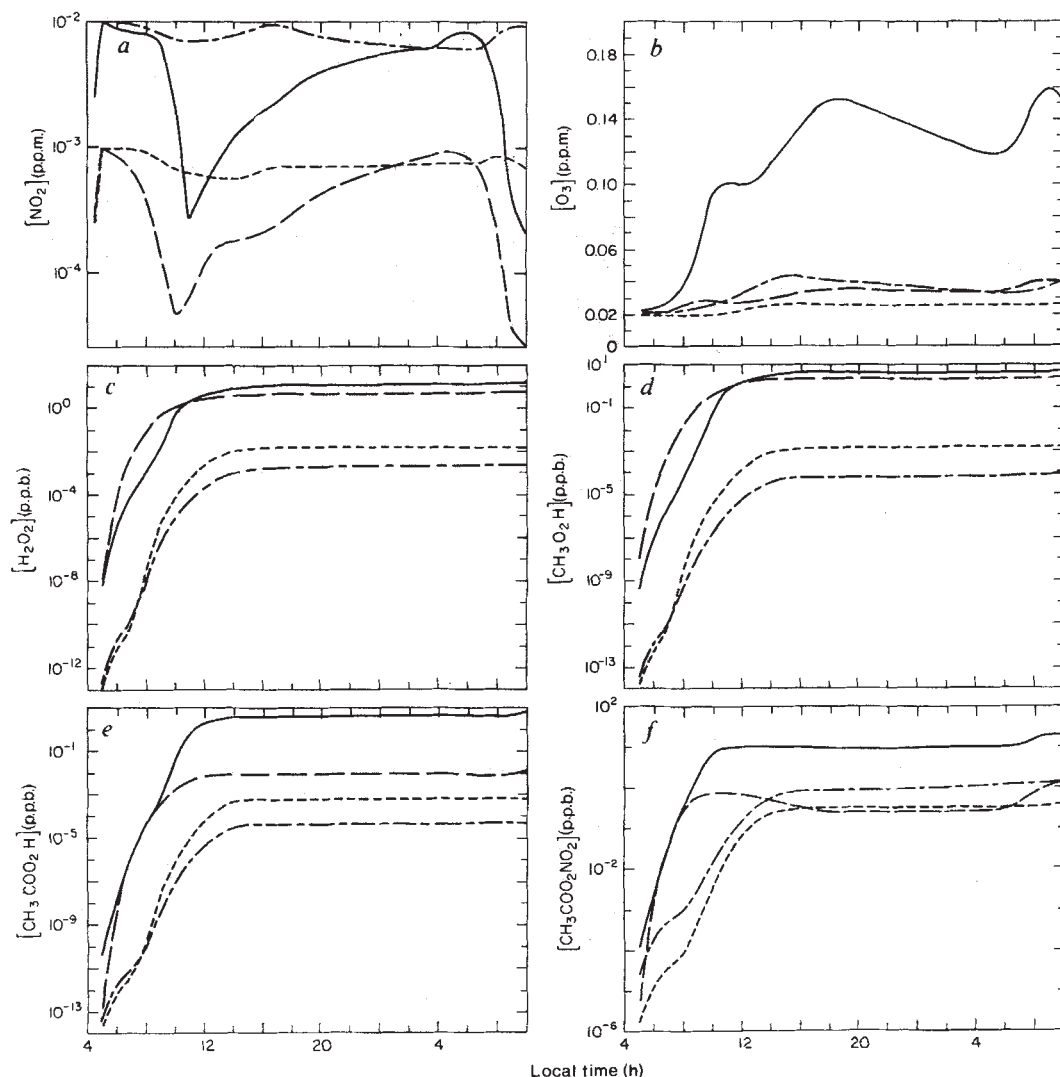


Fig. 7 Theoretical concentrations of various reactants and products as a function of time for the midsummer and midwinter scenarios I–IV of Table 1; lines are coded as outlined in Fig. 4 legend. *a*, NO_2 ; *b*, O_3 ; *c*, H_2O_2 ; *d*, $\text{CH}_3\text{O}_2\text{H}$; *e*, $\text{CH}_3\text{COO}_2\text{H}$; *f*, $\text{CH}_3\text{COO}_2\text{NO}_2$. Units are parts per 10^6 in *a* (p.p.m.) and parts per 10^9 in *b–f* (p.p.b.).

their 'standard model' for this case: solar zenith angle, 30° ; cloud transmissivity, 0.5; liquid water content of cloud, 1.5 g m^{-3} ; $[\text{O}_3]$, 30 p.p.b.; and temperature, 7°C . There are no reliable data related to the actual size of α available, but it seems reasonable to expect it to be $>10^{-3}$; if such is the case, this reaction pathway can be an important source of acid in the troposphere during daylight hours.

There are various other liquid-phase reactions that may contribute to S(IV) oxidation but which appear to be less important than the peroxides and ozone. The data of Penkett *et al.*³⁸ show that the uncatalysed oxidation of S(IV) by oxygen is unimportant for tropospheric conditions²⁶. However, Fe(III) and Mn(II) ions catalyse this oxidation so that this process can be an important source of acid for certain conditions (see Fig. 6, curves *c*, *d*). We have assumed a range of concentrations which we believe is typical of the rain water in the eastern United States: $[\text{Fe(III)}] = 10^{-5}$, $[\text{Mn(II)}] = 10^{-6} \text{ M}$ (curve *c*); $[\text{Fe(III)}] = 10^{-7}$, $[\text{Mn(II)}] = 10^{-8} \text{ M}$ (curve *d*). At the highest concentrations assumed here, the equivalent gas-phase rates of SO_2 conversion to acid can be $>100\% \text{ h}^{-1}$ in cloud water at $\text{pH} > 5$. These rates fall dramatically as the acidity of the cloud water increases: 100, 10 and $1\% \text{ h}^{-1}$ for pH values of 5, 4 and 3, respectively. For the conditions observed in Los Angeles fog water by Hoffmann and Jacob²¹ ($\leq 10^{-4}$ and 10^{-5} M for Fe(III) and Mn(II), respectively), this pathway of acid generation can dominate all others, especially during the night.

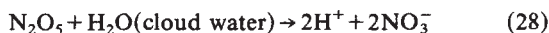
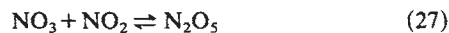
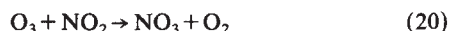
Oxidants such as peroxyacetyl nitrate and its higher homologues, even when present at 5 p.p.b. in the gas phase, are relatively unimportant oxidants for S(IV) (ref. 48) (see curve *m* for peroxyacetyl nitrate at 22°C).

It is clear from Fig. 6, curve *l*, that graphitic carbon (1 mg l^{-1} cloud water) is an unimportant catalyst for the S(IV) oxidation in clouds present in air masses with usual pollutant concentrations. We used the rate equations of Chang *et al.*⁴⁹. For the carbon catalysis to compete with H_2O_2 in solutions with pH values < 6 , its concentration must exceed 0.1 g l^{-1} , a value which is probably encountered rarely in the troposphere. In some cases a very high carbon content has been observed in precipitation^{50,51}, and graphite carbon-catalysed oxidation of bisulphite ions could be significant for these seemingly rare conditions. Nitrite ion rates of S(IV) oxidation also have been shown to be unimportant for the commonly encountered tropospheric conditions^{20,22,26,52}.

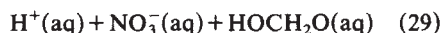
Simulations of the gas-phase chemistry for the scenarios I–IV reported here give some clues as to the expected concentrations of various oxidants that can be developed in theory for midsummer and midwinter conditions (Fig. 7). The estimates of the concentrations of $\text{CH}_3\text{O}_2\text{H}$ and $\text{CH}_3\text{COO}_2\text{H}$ are only qualitative indicators of these quantities as the rate constants for the formation reactions of these species ($\text{CH}_3\text{O}_2 + \text{HO}_2 \rightarrow \text{CH}_3\text{O}_2\text{H} + \text{O}_2$, $\text{CH}_3\text{COO}_2 + \text{HO}_2 \rightarrow \text{CH}_3\text{COO}_2\text{H} + \text{O}_2$) are uncertain. We have chosen the lower of current estimates for these reactions ($k = 1.5 \times 10^{-12} \text{ cm}^3 \text{ per molecule per s}$) (ref. 53). Also the neglect of loss processes at the ground in these simulations adds to the uncertainty in these estimates, but they should be qualitatively useful. In the case of H_2O_2 , $\text{CH}_3\text{O}_2\text{H}$ and $\text{CH}_3\text{COO}_2\text{H}$, the theoretical maximum concentrations can be well above the 1 p.p.b. level assumed for the rate calculations summarized in Fig. 6. Note also the striking difference between the concentrations of all the oxidants formed in summer and in winter. The

theoretical midsummer maxima (p.p.b.) for scenarios I and II, respectively, of Table 1 are: H_2O_2 , 12, 4.7; $\text{CH}_3\text{O}_2\text{H}$, 4.2, 2.0; $\text{CH}_3\text{COO}_2\text{H}$, 4.1, 0.008; and O_3 , 150, 35. For midwinter cases III and IV much lower values are seen: H_2O_2 , 0.002, 0.015; $\text{CH}_3\text{O}_2\text{H}$, 6.8×10^{-5} , 1.3×10^{-3} ; $\text{CH}_3\text{COO}_2\text{H}$, 4.4×10^{-5} , 6.1×10^{-4} ; O_3 , 44, 27.

Nitric acid generation in clouds. Comparisons of clear air composition with that of cloud water in November measurements suggested to Lazrus *et al.*⁵⁴ that nitric acid was produced in clouds. The most probable reactions involve the uptake of N_2O_5 and/or NO_3 into cloud water (see refs 55–58). The reaction sequence is the following:



The NO_3 radicals may also be captured by cloud water and react in part with soluble organic matter such as hydrated formaldehyde (HOCH_2OH) to generate nitric acid as well as oxidation products of the organic species in reaction (29):



The theoretical studies of Heikes and Thompson^{55,56} suggest that the rates of HONO_2 formation in clouds by reactions (28) and (29) and their analogues can be very significant (equivalent gas-phase conversion rates for NO_2 of hundreds % h^{-1}), provided that the sticking coefficients for N_2O_5 and NO_3 on cloud droplets or moist aerosol particles are $>10^{-3}$. The night-time generation of HONO_2 and nitrate aerosol (NH_4NO_3) in Los Angeles (Richards⁵⁸) and the depletion of NO_3 (and by inference, its coupled N_2O_5 storage source) in night-time atmospheres of high humidity or in the presence of clouds or rain^{36,59}, provide indirect field evidence for these reactions. In theory the N_2O_5 -cloud water reaction (28) can become highly important in winter when the lower temperatures favour the storage of the N_2O_5 product. The significant build-up of this compound can occur during the night (Fig. 5b), and HNO_3 can be formed as the N_2O_5 contacts clouds, fogs or moist aerosol surfaces common in the winter season. It seems probable that the diminished rate of HNO_3 formation from the $\text{HO}-\text{NO}_2$ reaction (14) during the winter months is supplemented by the N_2O_5 -cloud water reaction during this period. It is also probable that the relative insensitivity of the nitrate deposition data to season reflects the contribution of reaction (28). The SO_2 analogue to the reaction sequences (20), (27) and (28) cannot lead to the generation of sulphuric acid at night. The oxidation of SO_2 by O_3 in the gas phase is very slow¹⁹, and the midwinter rates of sulphate generation die with the decline in generation of HO , HO_2 and other oxidants.

Coupled gas/liquid acid generation

It is clear that the two most important oxidants for HSO_3 in cloud water, H_2O_2 and O_3 , arise in large part from gas-phase reactions. We have observed previously that the generation of ozone depends on a complex set of gas-phase reactions which are a function of the concentrations of NO_x , hydrocarbons and their oxidation products, the relative humidity and the sunlight intensity. In the gas phase, H_2O_2 arises from the interactions of hydroperoxy (HO_2) and hydrated hydroperoxy ($\text{H}_2\text{O} \cdot \text{HO}_2$) radicals:



The apparent second-order rate constant (k) for H_2O_2 formation ($R(\text{H}_2\text{O}_2) = k[\text{HO}_2]^2$) is very sensitive to the concentration of

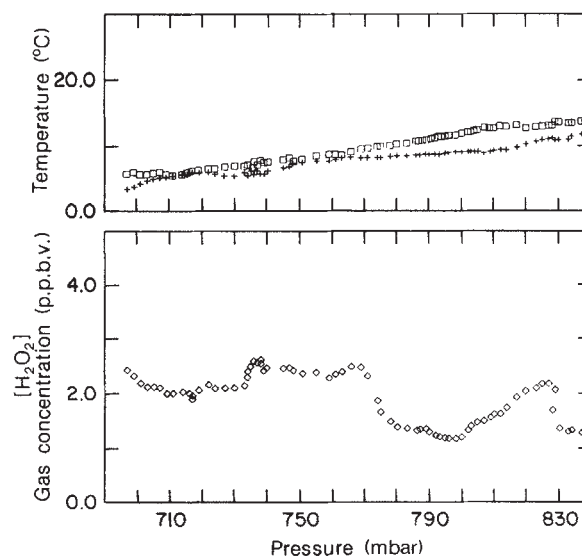


Fig. 8 Temperature (□), dew point (+) (upper part) and concentrations of gaseous H_2O_2 (◇) (in parts per 10^9 by volume, p.p.b.v.) (lower part) measured in a vertical profile over Elmira, New York (27 October, 1984, 1145–1230 h). The pressure range corresponds approximately to altitudes from 1,400 to 2,800 m above ground level.

water vapour; increasing the H_2O pressure from 0 to 14 torr doubles the constant⁶⁰. In theory other mechanisms also can contribute to H_2O_2 generation in the cloudy troposphere. For example, Zika *et al.*⁶¹ reported that the time dependence of $[\text{H}_2\text{O}_2]$ in rain during storms in southern Florida and the Bahamas did not follow that for SO_4^{2-} and NO_3^- in the rain water. They concluded that aqueous-phase H_2O_2 generation may be important. Chameides and Davis⁴⁷ have suggested from theoretical considerations that HO_2 radicals, generated in the gas phase, can be transported to the cloud water, and aqueous-phase reactions of HO_2 (and O_2^- derived from HO_2) can account for a significant rate of H_2O_2 formation in clouds.

Schwartz⁶² came to a similar conclusion from other theoretical considerations. Provided that the sticking coefficient of HO_2 radicals on cloud droplets is $>10^{-3}$, which seems likely, the rates of H_2O_2 generation by this coupled gas-phase cloud process are in theory approximately equal to those of the gas-phase sequence of reactions (32)–(34). Calvert *et al.*³⁶ and Hoffmann and Jacob²¹ have suggested an additional type of H_2O_2 -forming reaction: photosensitized generation at the surface of certain sunlight-absorbing semiconductor solids suspended in cloud water. These processes can only be important sources of H_2O_2 if rather substantial amounts of reasonably efficient sensitizers are present in the cloud water. The limited concentration data available for solid photosensitizers in precipitation suggest that this mechanism of H_2O_2 formation is only important in unusual circumstances. Kok has suggested that lightning is another potential source of H_2O_2 in clouds⁶³. However, the relatively large amounts of H_2O_2 predicted in our simulations (Fig. 7c) come entirely from the gas-phase processes (32)–(34), the only mechanism for H_2O_2 formation which was included in the reaction sequence chosen for the simulations.

One of the greatest uncertainties in the current theory of acid generation in the troposphere has been the magnitude of the available supply of H_2O_2 present in the atmosphere. In theory, H_2O_2 could be the limiting reagent for acid generation under many conditions. Recent measurements of the gas-phase $[\text{H}_2\text{O}_2]$ in the non-urban troposphere of the eastern United States have provided the first experimental estimates of this important parameter. We have used newly designed, aircraft-borne, H_2O_2 detection equipment^{64,65}. The flights were made during October and November 1984 in an instrumented aircraft (Beechcraft Queen Air). Significant levels of H_2O_2 were observed in most of the air masses sampled. Figure 8 shows a typical vertical profile

observed near Elmira, New York, on 27 October 1984 (1145–1230 h) for the gaseous H_2O_2 concentration together with the temperature and dew point of the air mass. Of course, theory suggests that the largest gas phase $[\text{H}_2\text{O}_2]$ will be observed in summer and the smallest in winter, with an intermediate concentration in the spring and autumn during which these first measurements were made. Observations of the $[\text{H}_2\text{O}_2]$ in mid-summer and midwinter air masses have not been made to date. However, the H_2O_2 present in winter precipitation at Whiteface Mountain, New York and Boulder, Colorado, is typically much smaller than that in summer samples. This is expected if the reactions of the HO_2 radicals, generated in reaction sequences which are initiated photochemically, are the dominant source of H_2O_2 . Our findings confirm that gaseous H_2O_2 is present in the troposphere during the autumn in sufficient quantity to be a major oxidant for S(IV) in cloud water, and it is a reasonable extrapolation of theory that gas-phase H_2O_2 will be more abundant during summer in the eastern United States.

Here we have shown that both gas and liquid-phase processes are important in producing acids in the troposphere. Although many attempts have been made to compare the extent to which each contributes to the annual deposition of acids, such results are highly speculative at this stage. The proper coupling of the chemistry of the solution and gas phases must be made in a much more sophisticated model before any such judgements can be meaningful.

Discussion

In view of the considerations given here, we suggest that the observed seasonal dependence of sulphate deposition in eastern North America, largest in midsummer, lowest in midwinter (Fig. 1), probably reflects the much greater rates of generation of oxidizing agents (such as HO radical, O_3 , H_2O_2 and $\text{CH}_3\text{O}_2\text{H}$) in summer than in winter. All of the oxidant-forming reactions are triggered by the HO radical formation involving the O_3 molecule [reaction (10)]. Note that the general shape of the yearly distribution of sulphate ion deposition shown in Fig. 1a matches well the shape of the maximum $J[\text{O}_3]$ values for each month (solid curves in Fig. 1a). This strongly suggests that both the gas- and liquid-phase chemistry of sulphuric acid generation are strongly influenced by photochemistry. On the other hand, there is no strong dependence of the nitrate ion deposition on season. We consider that this can be explained in terms of the changing contribution from the two major HNO_3 sources with season. In summer, nitric acid generation will probably be dominated by the fast reaction (14) with some acid generation in the reaction sequence (20), (27) and (28). In winter, when the $[\text{N}_2\text{O}_5]$ is maximal (see Fig. 5b), the HNO_3 generation in reaction (28) can become more important as reaction (14) decreases in importance. The strong seasonal variations in sulphate generation, coupled with the nearly constant nitrate generation during the seasons, leads to the observed strong seasonal dependence on the $\text{SO}_4^{2-}/\text{NO}_3^-$ molar ratio in deposition (Fig. 2a). The lack of strong dependence of the $\text{NH}_4^+/\text{SO}_4^{2-}$ molar ratio on season (Fig. 2b) suggests that the NH_3 sources must show a seasonal dependence which follows roughly that of sulphuric acid (sulphate). It is reasonable to suppose that emissions of ammonia during the summer (accompanying high biological activity) will exceed those of winter. However, existing measurements of the ambient $[\text{NH}_3]$ in the eastern United States are too sparse to test this suggestion.

The present report shows that current theories on acid development in the troposphere are in qualitative accord with the limited observations. The results suggest that the seasonal variation in sulphate deposition results largely from an oxidant limitation during the winter when photochemical activity is at its minimum. To what extent the summer acid deposition, the dominant deposition during the year, is oxidant-limited remains unclear. The very limited timescale of the record of acid deposition and emissions of precursors in eastern North America prevents any definitive conclusion. A committee of the US National Research Council recently studied the existing limited

field data related to acid deposition⁶⁶. They observed that there was a near equality between the yearly averaged $\text{SO}_4^{2-}/\text{NO}_3^-$ molar ratios in deposition over a large part of the eastern United States and the yearly averaged SO_2/NO_x molar ratios of emissions over the same large area. From this and other limited evidence they concluded that a uniform decrease in SO_2 emissions may result in a proportionate decrease in sulphate deposition when averaged over large areas and a period of a year. They assumed that factors such as meteorology, emission of oxides of nitrogen, hydrocarbons and basic materials (such as NH_3 , CaCO_3) remained unchanged during the period. Of course, a policy of uniform reduction of SO_2 from all sources in a large region of the eastern United States is not the most cost-efficient control strategy. However, in view of the relatively simplistic transport-deposition models available at the time of the review and the large uncertainties related to the predictions of air-mass motions, the committee concluded that the consequences for deposition in ecologically sensitive areas of changes in spatial patterns of emissions in eastern North America, such as reducing emissions in one area by a larger percentage than in other areas, could not be objectively predicted.

Although our knowledge of the chemistry of acid development in the troposphere is considerable, there are many remaining uncertainties which plague the development of sound control strategy. Decreases in the emissions of sulphur and nitrogen oxides will, of course, help alleviate the problem, but the extent of response in acid deposition at sensitive receptor sites to cutbacks in precursors at specific sources remains uncertain. Provided that we can afford to delay control measures for some years without disastrous results, an assumption which biologists should judge, then laboratory and field studies of the acid deposition problem, coupled with accurate model development, should provide the firmer evidence upon which to build cost-effective control strategies.

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ARTICLES

Global interactive transport simulations of nuclear war smoke

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Global circulation model simulations are reported in which smoke created by fires following a large nuclear war is allowed to move with the model winds. A substantial fraction of smoke generated over NATO and Warsaw Pact territories in July moves rapidly upward and across the Equator in less than two weeks, but the dispersal of smoke in January is less pronounced. Decreases in Northern Hemisphere land surface temperature are large in July, but less severe in January.

MASSIVE amounts of smoke from fires produced by a large nuclear war could create severe atmospheric and environmental effects over widespread areas^{1,2}, the principal effect of which might be a severe cooling over land areas caused by a decrease of solar energy reaching the surface. Major uncertainties in the problem of 'nuclear winter' concern the total quantity of smoke produced, the scale of the spread of such smoke, and the rate of removal of smoke from the atmosphere by cloud and precipitation processes³. Some commonly acknowledged uncertainties can only be narrowed by recourse to global general circulation models (GCMs), for example, the extent and rapidity of smoke spread from regions of generation, the time-dependent geographical patterns of surface effects, the importance of the initial height of injection of smoke and the modulation of effects by season. Here I address these uncertainties. (The lifetime of widespread smoke in the atmosphere and the long-term effects of remnant smoke and dust in the upper troposphere and stratosphere (8-50 km) are not addressed here.)

The possible atmospheric effects of nuclear war have been discussed recently by the National Research Council (NRC)³. Previous multi-dimensional global models of nuclear winter effects have made use of a 'fixed' smoke assumption in which smoke could affect the atmospheric circulation but was not itself moved by the simulated winds⁴⁻⁸. It is more realistic to allow smoke to move interactively with the atmosphere. Aleksandrov⁹ and MacCracken and Walton¹⁰ have independently used two-layer, three-dimensional global circulation models in preliminary smoke transport experiments. The two-layer models indicate that smoke can reach the Southern Hemisphere, but it is not clear to what extent this result is caused by the limited vertical resolution of the models. Haberle *et al.*¹¹ have shown with a two-dimensional circulation model of good vertical and

latitudinal resolution that a significant fraction of nuclear war smoke could reach the stratosphere. The two-dimensional model did not find that substantial amounts of smoke would move towards the Equator. Malone *et al.*¹² have recently performed smoke transport simulations with a fully three-dimensional model including an approximation for smoke removal by precipitation, and find that heating of the smoke by absorption of solar energy promotes both upward and equatorward smoke movement that does not occur in calculations using transparent smoke.

This study extends the model of Covey *et al.*^{4,5} to the interactive transport case. In particular, several of their results are re-examined in the light of more comprehensive simulations: (1) can a massive smoke cloud in Northern Hemisphere mid-latitudes in summer produce a circulation having a strong tendency to move smoke upward and equatorward; (2) can mid-latitude land surface temperatures under a dense smoke cloud drop rapidly by an average of ~25 °C in July, but much less in January; (3) is the pattern of land surface temperature change heterogeneous, with greater average cooling in mid-continent and much less along coasts; (4) can heating of the atmosphere by smoke reduce condensation and precipitation in the smoke cloud, thus reducing smoke scavenging and increasing smoke lifetime? The answer to each of these questions is 'yes', as explained below.

The model

A description of the GCM used in this study, the National Center for Atmospheric Research (NCAR) Community Climate Model, is given elsewhere^{13,14}. Most of the assumptions and approximations, except for transport additions described below, are the same as those used by Covey *et al.*, for example, the

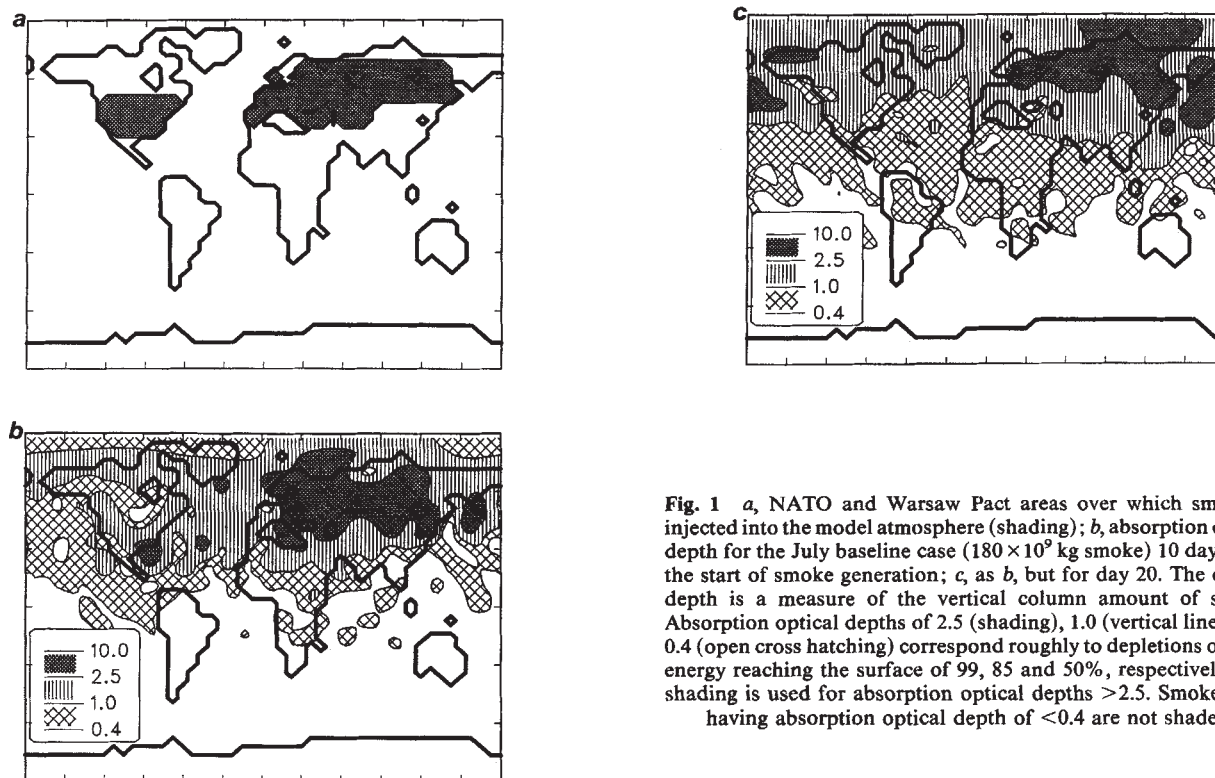


Fig. 1 *a*, NATO and Warsaw Pact areas over which smoke is injected into the model atmosphere (shading); *b*, absorption optical depth for the July baseline case (180×10^9 kg smoke) 10 days after the start of smoke generation; *c*, as *b*, but for day 20. The optical depth is a measure of the vertical column amount of smoke. Absorption optical depths of 2.5 (shading), 1.0 (vertical lines) and 0.4 (open cross hatching) correspond roughly to depletions of solar energy reaching the surface of 99, 85 and 50%, respectively. The shading is used for absorption optical depths >2.5 . Smoke areas having absorption optical depth of <0.4 are not shaded.

simulated smoke put into the NCAR model is assumed to be non-scattering (only absorptive) in solar wavelengths and to have no direct interaction with infrared radiation. The inclusion of dust and smoke scattering in the radiative calculations, given the same absorption optical depth, would act to reduce further the solar energy available at the surface, but would also reduce the solar energy absorbed in the smoke cloud. Neglecting the infrared opacity of the smoke scenario used here has been shown to be a reasonable first approximation¹⁵ for surface-temperature calculations, but may lead to some error in heating rates for smoke in the stratosphere. The atmosphere and surface are represented by $\sim 4.5^\circ$ latitude and 7.5° longitude resolution, with nine layers throughout the troposphere and stratosphere from the surface to ~ 30 km altitude.

Various versions of the model produce generally reasonable simulations of temperature and wind fields under normal January and July conditions¹⁶ and simulations of the complete annual climatic cycle^{17,18}. The model is verified for climatic change by testing with large forcings of known magnitude and climate effect: the natural annual and diurnal cycles of solar-energy forcing. The annual cycle of climate change at temperate and polar latitudes is a much larger change than any plausible nuclear winter effect. GCMs in general have shown that the geographical distribution and magnitude of the annual temperature cycle can be well simulated^{17,19}. The diurnal cycle of solar forcing is comparable in magnitude and timescale to solar-energy changes that might occur under dense smoke clouds. Simulations with an experimental version of the NCAR GCM having diurnal cycle capability show that it is possible to produce a reasonable simulation of diurnal temperature variations (R. E. Dickinson, personal communication).

Smoke is transported in the model in the same way as water vapour¹⁴. The spectral advection scheme used in the NCAR model is not well suited to tracer studies and is augmented here to correct problems of negative concentrations and rapid dispersal of small positive concentrations. A correction procedure is adopted that tends to damp 'small' negative or positive values of smoke concentration to zero, while leaving the main mass of smoke unaffected. 'Small' concentrations are those less than a threshold value that is height and time dependent and defined as 10% of the maximum smoke concentration at each vertical

level of the model. The smoke-mixing ratios are multiplied at each model time step by a damping factor of 0.9 for negative concentrations and 1.0 for mixing ratios above the threshold (that is, no damping). The damping factor varies linearly with mixing ratio between the two limiting cases. This *ad hoc* procedure successfully controls noise but does not guarantee mass conservation. As a final step, the global smoke mass is corrected by multiplying the smoke concentration at every grid point by the ratio of global smoke mass before and after each threshold damping procedure. The modified and original model transport schemes were compared to ensure that the modifications had little effect on the bulk movement of smoke. A more appropriate flux-corrected transport²⁰ formulation will be included in the future, but the present formulation is certainly sufficient to support the conclusions reached below. A comprehensive simulation would include the removal of smoke from the atmosphere as well as its injection and transport. The simulations do not yet include smoke removal, which is primarily associated with water condensation and rainout. The incorporation of smoke removal is currently being investigated.

A baseline smoke scenario is adopted in which 180×10^9 kg smoke (the baseline smoke concentration suggested by the NRC report³) is introduced over selected NATO and Warsaw Pact territories in July (Fig. 1*a*). Smoke is injected at a constant rate over 2 days with uniform mixing ratio between the surface and an altitude of 7 km. The model is free to move smoke away from the regions of generation at any time during the injection period and thereafter. The simulation procedure involved running the model under normal conditions for a period of time, then at the initial perturbation time (day 0) a large-scale smoke introduction is started in the specified volume of atmosphere. It would be more realistic to introduce the smoke as plumes over many probable fire locations. Mesoscale circulations would transport, scavenge and disperse the dense plumes until they merged into the large 'patches' that the global NCAR model can actually resolve. The coarse grid of the model limits the geographical resolution of the smoke source regions to a 10^3 km scale. The fate of nuclear fire smoke in the first few days after its generation is a problem suited more to mesoscale meteorological models (10 – 10^2 km grid scale) than to coarse global models.

Results

The location of smoke 10 days after the start of smoke generation is shown in Fig. 1b. The plotted quantity is absorption optical depth proportional to the local vertically integrated column amount of smoke. Absorption optical depths of 2.5, 1.0 and 0.4 (see Fig. 1 legend) correspond roughly to depletions of solar energy reaching the surface of 99, 85 and 50%, respectively. By day 10, smoke has spread around the Northern Hemisphere into the Southern Hemisphere, but remains inhomogeneous. Smoke spread has increased by day 20, the last day of the July baseline simulation (Fig. 1c).

Covey *et al.*⁴ suggested that large concentrations of smoke in the presence of strong solar-energy forcing would distort the normal north-south atmospheric circulations so that smoke would be moved southwards more rapidly than normal. This more rapid movement is confirmed by comparing the July baseline simulation with a control simulation using transparent, but mobile smoke. By day 15, <1% of the control-case smoke is in the Southern Hemisphere, compared with 11% for the baseline case. Remarkably similar results for smoke spreading rate have been produced by models with a wide range of vertical resolutions from 2 levels¹⁰ to the 9 levels used here to 20 levels¹². Present simulations of global smoke transport do not seem to be very sensitive to the vertical resolution of the model used.

Figure 1 suggests that smoke tends to accumulate preferentially over the Eurasian land mass. This would be a significant result, if confirmed, because nuclear winter effects that would be asymmetrical between the major world powers could conceivably create perceptions of relative vulnerability or advantage²¹. However, the results in Fig. 1 reflect the fact that the initial smoke injection over Eurasia is larger than that over North America. Smoke generation is simply assumed to be constant per unit area, and the assumed Eurasian target area is larger than the corresponding area in North America. Other scenarios, short of detailed targeting lists, seem no less arbitrary. A simulation using an unbiased zonally symmetrical injection of smoke shows no tendency for the smoke to accumulate preferentially over any continental region.

The vertical movement of the smoke is illustrated in Fig. 2. By day 10 (Fig. 2a) a substantial quantity of smoke has risen well above the injection altitude range of 0–7 km and a 'tongue' of smoke between 10 and 20 km altitude is seen projecting into the Southern Hemisphere to ~20° S. On day 20 the upwards and southwards spread of the smoke has increased, with smoke extending to 30° S (Fig. 2b). The rapid vertical movement of the smoke in the July baseline case is a direct consequence of intense solar heating of the smoke cloud, that is, the smoke is self-lofting. Thirty-one per cent of the smoke is above 10 km altitude on day 15 in the baseline case, compared with 6% for the control case using transparent smoke.

The simulated vertical movement of smoke is caused only by large-scale motions explicitly resolvable by the model. In addition to such resolvable motions, so-called sub-grid-scale motions too small to be simulated explicitly would tend to mix the smoke. An unstable strongly heated layer forms at the top of the smoke cloud in the baseline simulation. This layer would be a site of strong dry convection (but probably little moisture condensation) that would tend to mix the smoke upwards and increase the rapidity of its vertical movement¹¹.

The solar heating of the smoke has profound consequences for the structure of the atmosphere and probable smoke lifetimes. Normally, the tropopause (the boundary between the mixed troposphere and more stable stratosphere) is at an altitude between 10 and 18 km. Solar heating rates of the order of 10 °C per day at the top of the smoke cloud in the Northern Hemisphere baseline case force the effective tropopause downwards to between 5 and 8 km altitude ~1 week after the start of smoke injection. Moisture condensation necessary for effective smoke removal is virtually eliminated above 5 km. However, smoke that remains at low altitudes for more than a few days would have a good chance of being removed by the remaining cloud scavenging processes. The fraction of smoke that would be

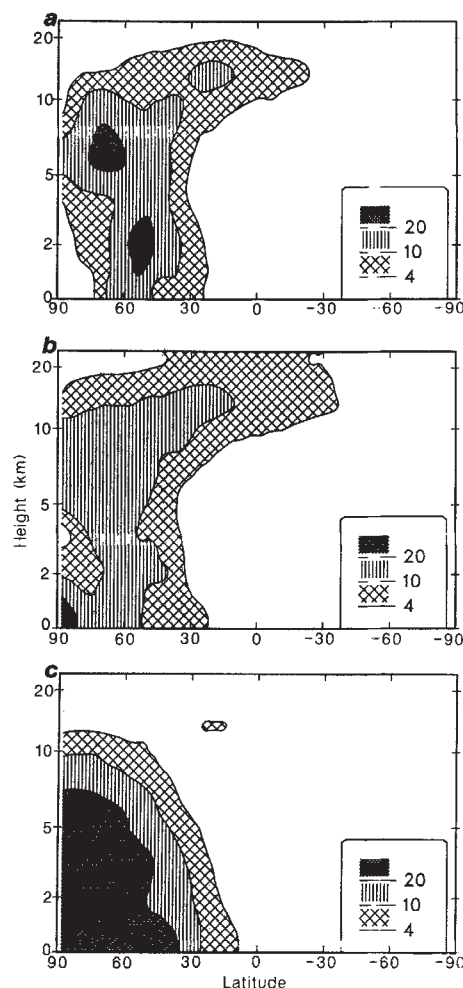


Fig. 2 The mixing ratio of smoke ($\times 10^{-8}$ g per g, or mass of smoke per mass of atmosphere) averaged over longitude and displayed as a function of latitude and height. *a*, July baseline smoke case, day 10; *b*, July baseline smoke case, day 20; *c*, January smoke case, day 30. Denser shading patterns indicate higher smoke concentration. Mixing ratios $>20 \times 10^{-8}$ are shown as the most shading; those of $10\text{--}20 \times 10^{-8}$ as vertical lines; those of $4\text{--}10 \times 10^{-8}$ as open cross-hatching; and those $<4 \times 10^{-8}$ are not shown. All smoke is injected between 0 and 7 km altitude.

removed on the normal smoke scavenging timescale of ~1 week²², and the fraction that would have a lifetime of weeks to months depend on the initial injection altitudes of the smoke and the rapidity of self-lofting. For the July baseline case described here the amount of long-lived smoke might be <50% of the assumed smoke injection. Malone *et al.*¹² reach similar conclusions based on the results of a model with explicit smoke removal.

The effects of the smoke on temperature at the surface are shown in Fig. 3. Biological effects would, of course, be severe if sub-freezing temperatures were to occur in the northern temperate latitudes in July. But even temperatures as 'mild' as 10 °C would be damaging in low-latitude regions not accustomed to low temperatures²³. The July surface temperature just before the start of smoke generation is shown in Fig. 3a. Subfreezing temperatures in the Northern Hemisphere are confined to the areas bordering the Arctic Ocean. By day 10 subfreezing temperatures are widespread in Eurasia and North America (Fig. 3b). Note that the relatively warm area in the interior of North America corresponds to a region of low smoke density in Fig. 1b. The correspondence between smoke and surface temperature implies that the surface responds to solar energy changes on a timescale of a few days, as noted by Covey *et al.*⁴.

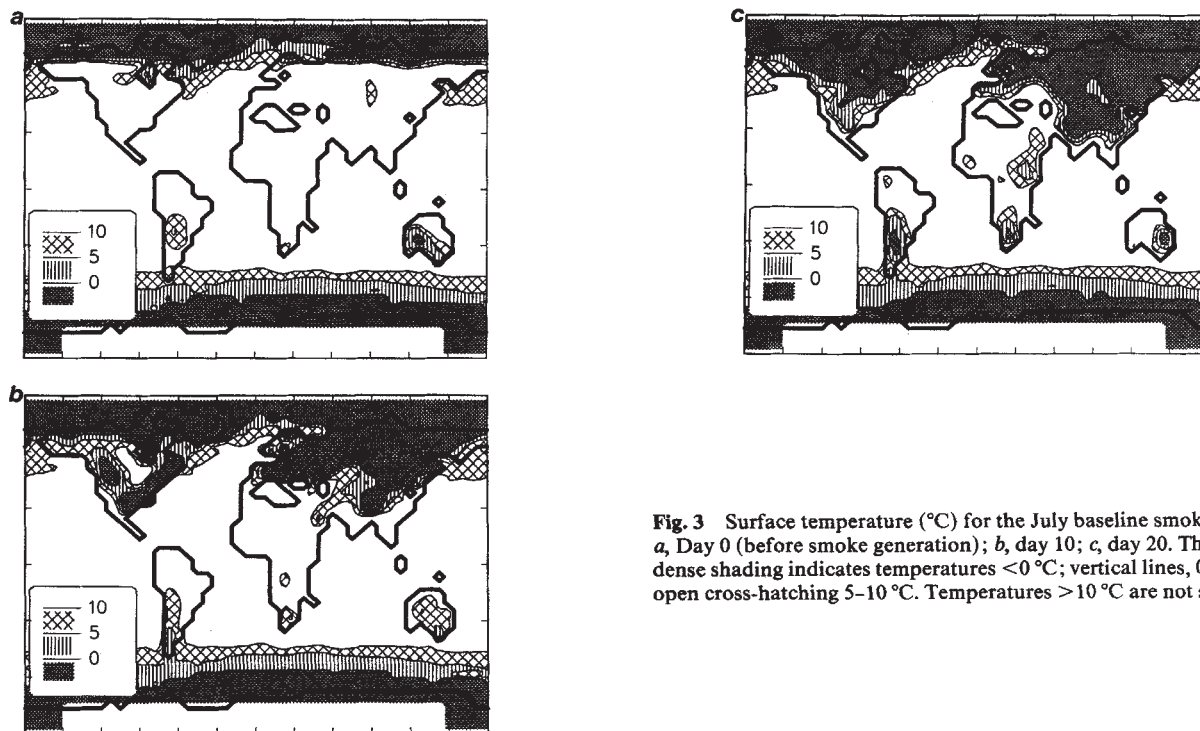


Fig. 3 Surface temperature ($^{\circ}\text{C}$) for the July baseline smoke case. *a*, Day 0 (before smoke generation); *b*, day 10; *c*, day 20. The most dense shading indicates temperatures $<0^{\circ}\text{C}$; vertical lines, $0-5^{\circ}\text{C}$; open cross-hatching $5-10^{\circ}\text{C}$. Temperatures $>10^{\circ}\text{C}$ are not shown.

The temperature distribution on day 20 is given in Fig. 3c. The temperature pattern is changed from day 10, especially over North America, East Africa and East Asia. The inhomogeneous distribution and time variation of cold temperatures are a combined consequence of a patchy smoke distribution and normal day-to-day weather variability. The differences between Fig. 3a and b are an indication that the quantitative details of the temperature distributions shown should not be taken too literally. Regional details and the time sequence of events would differ if the model were started with the weather conditions of a different July day, but the large signal resulting from nuclear smoke would still stand out clearly above normal weather noise. An ensemble of many simulations starting with different initial conditions could be used to establish weather statistics, but such an expensive undertaking is not justifiable given the status of present models. In addition, many simplifying assumptions have been made in the calculations that could modify the surface-temperature estimates, for example, the use of non-scattering smoke and the absence of a detailed simulation of the structure of the lowest few kilometres of the atmosphere. The most restrictive assumption may well be the neglect of smoke removal. This assumption tends to make the temperature decreases described in Fig. 3 more severe on average than those actually expected, especially for day 20.

Covey *et al.*⁴ suggested from their results that the spread of smoke in January would be less than in July. A January case will be considered to examine the sensitivity of the simulation to the assumed season of a war. All parameters are identical to the baseline July case except the season and length of simulation (30 days instead of 20). The zonally averaged mixing ratio of smoke on day 30 of the January case is shown in Fig. 2c. Even after 30 days the smoke has not spread upwards or southwards very much on average from the initial area of injection. Instead, the smoke is largely contained in a region of the winter troposphere and tends to become well mixed in the absence of removal processes. Six per cent of the smoke exceeds 10 km altitude by day 15, compared with 1% for the January control case and 31% for the July baseline case. On the same day, 1% of January smoke is in the Southern Hemisphere, compared with $<1\%$ for the January control and 11% for the July baseline. The difference between January and July is the lack of strong solar heating to lift the smoke in January.

Covey *et al.*⁴ speculated that patches or streamers of smoke could quickly reach the Southern Hemisphere in January despite the absence of a large change in Northern Hemisphere circulation, but this effect is not found here in the January case. Patchiness in either January or July is not as pronounced as once thought⁴. Vertical shear of horizontal winds tends to spread smoke efficiently horizontally at altitudes above 5 km. This upper troposphere smoke can obscure lower altitude patchiness that might arise from local precipitation and smoke removal.

The dramatic difference in surface-temperature response between January and July is shown in Fig. 4. Large areas of the Northern Hemisphere experience time-averaged temperature decreases exceeding 15°C in July (Fig. 4a). Allowing the smoke to move out of the target latitude zone produces temperature effects about as severe in magnitude as those found by Covey *et al.*⁴, but substantial effects occur over a wider area. In contrast, the January case (Fig. 4b) shows surface temperatures rarely decreasing by more than 15°C on average. Only in the latitude belt $20-35^{\circ}\text{N}$ do the temperature change averages seem to be significant given the normal variability of the model. In this zone the mean temperature drop over land is $\sim 5^{\circ}\text{C}$. Normal observed winter temperature minima for many locations in this latitude band approach 0°C but have rarely, if ever, been below freezing. The time-averaged January land temperature at $20-35^{\circ}\text{N}$ is 4°C in the model, with sub-freezing temperatures confined to the northern margin of the zone. A 5°C temperature shift, although apparently small, could combine with normal weather variability to produce unusually low, damaging temperatures in the Northern Hemisphere winter subtropics.

In addition to testing the sensitivity of the simulations to season, it is important to examine the sensitivity of the results to at least two other parameters: the injection height and total amount of smoke. The injection height of the smoke controls the length of time the smoke may be subject to precipitation scavenging and may affect the circulation perturbation that lofts and transports the smoke in summer. A July low injection case was performed in which the smoke was placed in the model between 0 and 2 km altitude. This height range is unrealistically low for large fires³, but will serve as a large change from the baseline case. It is found that the low altitude of injection does not change the tendency of smoke to move upwards and southwards. The main difference between the two cases is a delay of

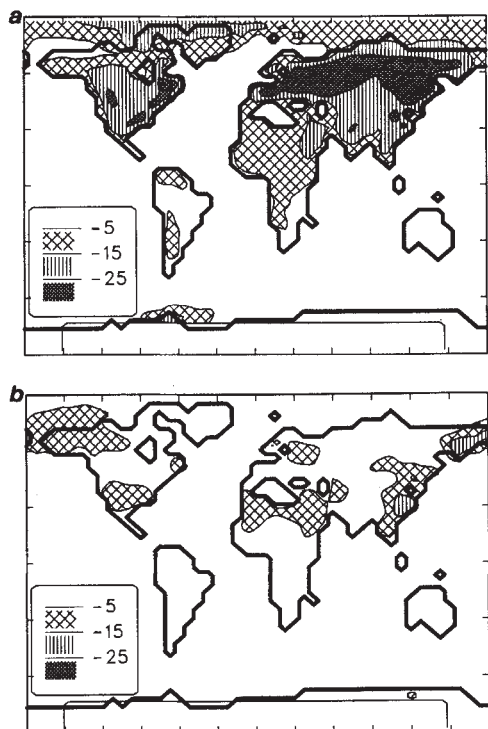


Fig. 4 The time-averaged change in surface temperature ($^{\circ}\text{C}$) shown as results from a smoke case minus a smoke-free control case. *a*, July case, days 5–20; *b*, January case, days 5–30. The most dense shading indicates temperature decreases of $>25^{\circ}\text{C}$; vertical lines, $15\text{--}25^{\circ}\text{C}$; open cross-hatching $5\text{--}15^{\circ}\text{C}$.

~ 5 days in the time it takes the low injection smoke to reach a given height or latitude compared with the baseline case. The extra time at low altitude is not very long, but could result in substantially more smoke removal than in the baseline case, if removal were to be included.

Smoke amounts of 60×10^9 and 540×10^9 kg are still within the uncertainty limits suggested previously³. These smoke amounts are used in July simulations. Averaged over days 10–15, the 60×10^9 kg case produces a land average surface-temperature decrease over $30\text{--}60^{\circ}\text{N}$ of 14°C compared with 23°C in the baseline case. The 540×10^9 kg case produces a 26°C decrease for the same time and area, indicating that adding more smoke would result in little additional cooling in the target latitude zone. However, very large injections of smoke are important for latitude zones outside the target area. The 540×10^9 kg case gives a 19°C decrease for land between 0 and 30°N on days 10–15, compared with 12°C for the baseline case and 4°C for the 60×10^9 kg case. The rate of spread of the smoke is nearly the same for these three July cases. However, there does seem to be a small self-shielding effect that makes the 540×10^9 kg smoke

case disperse more slowly than the 60×10^9 kg case. The 60×10^9 kg case allows solar energy to penetrate further into the smoke cloud, and therefore lift a larger fraction of the smoke mass. By day 15, 34% of the smoke of the 60×10^9 kg case is above 10 km altitude compared with 25% of the 540×10^9 kg case.

Conclusions

Global smoke transport simulations were performed using smoke injections over NATO and Warsaw Pact territories in July and January. Suggestions that smoke from a nuclear war could spread to non-combatant areas in the tropics and Southern Hemisphere, and that the atmospheric effects of smoke could act to increase the smoke lifetime, are generally supported by the July simulations presented here. The heating of smoke in July produces a self-lifting effect that rapidly moves a substantial fraction of the smoke upwards and a smaller fraction across the Equator in less than 2 weeks. The upwards and southwards spread of smoke in January is much smaller than in July because of weaker solar heating. In July, widespread large temperature decreases could occur over Northern Hemisphere land areas, and some environmental effects could extend to the mid-latitudes of the Southern Hemisphere a few weeks after a large nuclear war in which Northern Hemisphere cities were targeted. Surface-temperature effects in January are less pronounced, but biologically harmful temperature decreases could occur in the Northern Hemisphere subtropics.

Some major uncertainties in the nuclear winter problem that are unresolvable by global scale models will need to be reduced before substantially stronger statements about nuclear winter effects can be made, especially the amount of smoke produced by a given war scenario and the amount of smoke removed promptly in clouds associated with large fires. Moreover, as the principal impact of a nuclear winter would be biological, reducing the uncertainty in the estimates of biological effects of various climate response scenarios deserves a high priority. Further improvements that will enhance the credibility of the global models will include interactive removal of smoke, simulation of the diurnal and annual cycles of solar energy forcing and simulation of the long-term effects of remnant smoke and dust in the stratosphere. At this time the range of nuclear winter uncertainties permits a spectrum of effects from mild local cooling to subfreezing temperatures on a global scale. Of course, not all the possible scenarios in this range are equally probable, but even severe hemispheric-to-global-scale environmental effects remain a plausible outcome of a large nuclear war.

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Spatial and temporal patterns of *Krüppel* gene expression in early *Drosophila* embryos

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The Krüppel (Kr) locus is a member of the 'gap' class of segmentation genes of Drosophila melanogaster. Mutations at the Kr locus cause the deletion of contiguous segments from the embryonic body pattern. We have elucidated the spatial and temporal characteristics of Kr gene expression during early embryo development, the localization of cytoplasmic Kr⁺ activity and its spatial requirement for normal segmentation.

DETERMINATION of the segmental pattern in the *Drosophila* larva occurs at the blastoderm stage of embryogenesis¹⁻³. Many of the genes involved in the establishment of segmentation have been identified on the basis of mutations, and the cuticular phenotypes of the typically lethal mutant embryos have allowed the classification of the segmentation genes and the assignment of the domain of action for each of them⁴⁻⁷. Mutations of the gap class of segmentation genes result in deletions of contiguous segments in defined regions⁷. *Kr* is required specifically for thoracic and anterior abdominal segmentation⁷⁻¹⁰. Embryos homozygous for strong (amorphic) *Kr* alleles show a deletion of all three thoracic and five abdominal segments that are replaced by a partial duplication of posterior abdominal segments with reversed polarity⁷⁻¹⁰. In addition to the segmentation defect, the malpighian tubules are absent⁹. The phenotypes of a series of hypomorphic *Kr* alleles show progressively smaller gaps in the segmental pattern, with the weakest lacking meso- and metathorax and one abdominal segment^{8,10}.

That *Kr*⁺ gene requirement for normal segmentation is purely zygotic and is limited to the embryo period from blastoderm to extended germ-band stage has been inferred from combined morphological and genetic studies^{7,10} and from the developmental profile of *Kr*⁺ transcripts⁸. Here, we describe *in situ* hybridization experiments showing the spatial and temporal patterns of *Kr* gene expression in wild-type embryos and cytoplasm injection experiments that reveal localization of *Kr*⁺ activity in wild-type embryos and the spatial requirement for *Kr*⁺ activity in *Kr* embryos. We relate these experiments to *Kr* mutant phenotypes and to other genes involved in the establishment of the embryonic body plan.

Kr⁺ probe

The *Kr* locus is the source of several developmentally regulated transcripts ranging in size from 2.5 to >4 kilobases (kb) (U.B.R. *et al.*, in preparation). Together they represent <0.01% of poly(A)⁺ RNA⁸. The most abundant transcripts of 2.5 and 2.8 kb have been detected previously as a single broad band on Northern blots⁸; each of the other transcripts is ~50 times less abundant. A complementary DNA clone with sequences homologous with all these transcripts¹¹ has been isolated and used to prepare strand-specific ³H-labelled RNA probes (see Fig. 1 legend). These probes were hybridized to frozen sections of *Drosophila* embryos^{12,13} and sites of hybridization corresponding to *Kr*⁺ transcript accumulation were detected by autoradiography.

First *Kr*⁺ transcription

During the early cleavage stages, the *Drosophila* embryo develops as a plasmodium. After seven rapid nuclear divisions in the central yolk, most nuclei begin to migrate to the periphery

of the egg. (Yolk nuclei remain in the central region.) The first cells appear at the posterior end of the embryo (pole cells) after the ninth nuclear division. During the syncytial blastoderm stage that follows, nuclei at the periphery undergo four additional synchronous divisions. Following the 13th nuclear division, the ~6,000 peripheral nuclei elongate perpendicularly relative to the egg surface. About 30 min later, cell membranes grow inward from the oolema to enclose the nuclei, thus forming a single-layer epithelium, the cellular blastoderm, at ~3.5 h after fertilization¹⁴⁻¹⁶.

Transcripts of the *Kr* gene are first detected in syncytial blastoderm stage embryos after the 11th nuclear division. At this time, a specific hybridization signal occurs over a circumferential band of 8-10 nuclei in a region ~45-55% of the egg length from the posterior pole (Fig. 1a). We term this zone the *Kr*⁺ central domain. By the completion of the 13th nuclear division, a much stronger hybridization signal extends over the cytoplasm of 12-14 peripheral nuclei and the cytoplasm of yolk nuclei in the corresponding region of the embryo (Fig. 1b).

During the cellular blastoderm stage, *Kr*⁺ transcripts are still present in the *Kr*⁺ central domain in a band of 12-14 cells and the yolk cells contained within it. A new region of *Kr* gene expression also appears late in this stage in the posterior cap (~10 cells wide), excluding the pole cells. The signal intensity of this posterior *Kr*⁺ domain is at first weak, but increases significantly towards the end of the blastoderm stage. Concomitantly, *Kr*⁺ transcription decreases in the yolk but not in peripheral cells of the *Kr*⁺ central domain (Fig. 1c).

Spatial pattern of gene expression

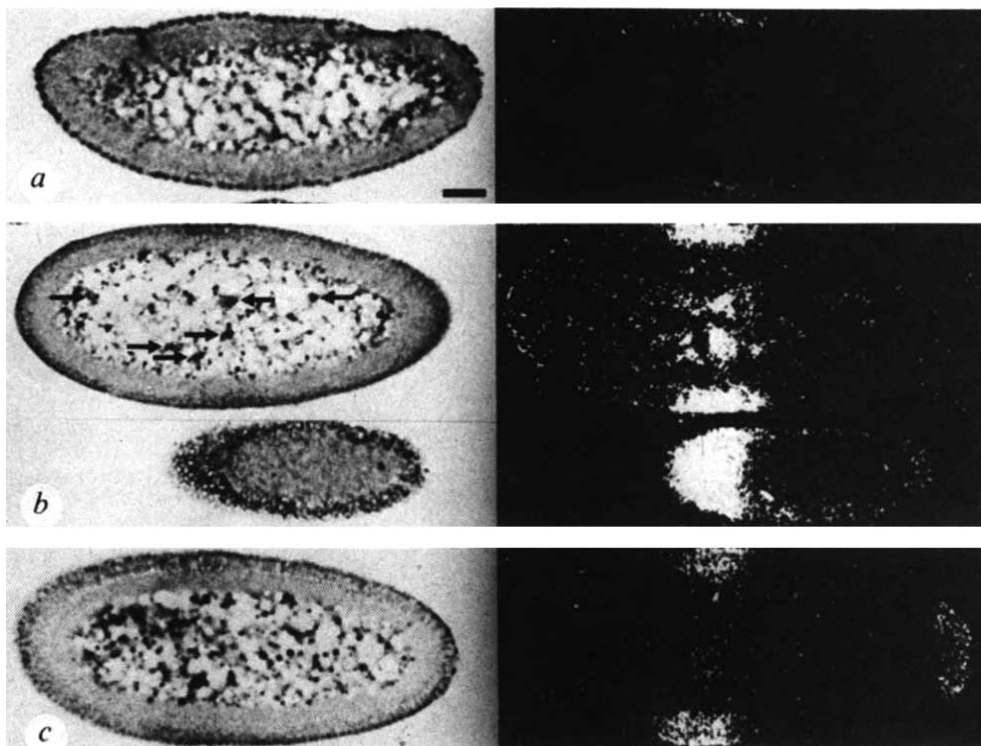
After the blastoderm stage, gastrulation is initiated by the invagination of cells, which subsequently form the mesoderm, along the ventral midline. The cephalic furrow simultaneously forms laterally about two-thirds of the distance from the posterior end, and cells forming the posterior polar plate begin to migrate dorsally¹⁴⁻¹⁸.

The spatial pattern of *Kr*⁺ transcript accumulation becomes more complex with the onset of gastrulation. First, a third zone of *Kr* gene expression develops suddenly in the anterior region of the embryo. This *Kr*⁺ anterior domain comprises a band of cells lying about six cell diameters anterior and parallel to the cephalic furrow (Fig. 2a). The signal intensity in this zone is less than 10% of the *Kr*⁺ central domain, which continues to comprise a band of 12-14 cells starting about 6-9 cells posterior to the cephalic furrow, and includes cells that have invaginated to form the mesodermal anlage (Fig. 2a). The ventral region of the *Kr*⁺ central domain appears substantially wider than the dorsal region, resulting from compression of dorsal cells and stretching of ventral cells at the beginning of and during germ-band extension¹⁴⁻¹⁷. With the onset of gastrulation, the signal intensity of the *Kr*⁺ posterior domain (which has begun to migrate dorsally) has further increased (Fig. 2a, b).

The restriction of *Kr*⁺ transcripts to the three *Kr*⁺ domains ends shortly after the beginning of gastrulation. The first depar-

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Fig. 1 Localization of Kr^+ transcripts in sections of blastoderm-stage *Drosophila* embryos. Bright-field photomicrographs of embryo sections (left) showing details of cellular organization and corresponding dark-field photomicrographs of the same sections (right). Silver grains developed in the autoradiographic emulsion appear as bright spots in dark-field optics. All sections are in the same orientation, anterior (left) and posterior (right). Scale bar, 50 μ m. **a**, Sagittal section of a syncytial blastoderm-stage embryo after the 11th nuclear division showing the first appearance of Kr^+ transcripts over peripheral nuclei ~45–55% of egg length. **b**, Horizontal section of an early cellular blastoderm-stage embryo with focus on yolk nuclei (arrows). Inset, tangential lateral section (including the Kr^+ central domain) indicating that Kr^+ gene expression is in a circumferential band of cells. **c**, Horizontal section of a late cellular blastoderm stage embryo showing the first appearance of Kr^+ transcripts in the posterior domain.



Methods. Sections of devitelinated embryos and subsequent treatments were done as described by Akam and Martinez-Arias²⁶. Strand-specific ³H-labelled RNA probes were synthesized as described with SP6 polymerase, linearized vector template and appropriate concentrations of ³H-UTP and cold nucleotides²⁷ to a specific activity of 6×10^7 d.p.m. μ g⁻¹ and a mean fragment length of 200 nucleotides. Plasmid template pSPcK6 (ref. 11) was used to prepare antisense RNA probe for localization of Kr^+ transcripts. Probes for determination of background for precise localization of the Kr^+ domains and for sensitivity of the Kr^+ probe relative to *ftz* gene expression (data not shown) were prepared similarly from pSPcK5 (ref. 11) and pSPF1 (the *ftz*⁺ probe). Sections were hybridized for 24 h at 44 °C at a final probe concentration of 1 μ g ml⁻¹ in hybridization buffer without dextran sulphate. After hybridization, the slides were treated with RNase A²⁶ and washed as follows before dehydration, autoradiography and staining by standard methods²⁸: 3×21 (4 $\times$)SSC for a total of 12 h (22 °C), 2×21 (2 $\times$)SSC for a total of 8 h (22 °C), 21 (0.2 $\times$)SSC for 15 min (50 °C) and 21 (0.2 $\times$)SSC for 15 min (22 °C). Autoradiographic exposure was 21 days at 4 °C. Orientation of embryos was determined in serial sections. Early embryos were staged by developmental time and by counting the number of peripheral nuclei^{14,19} in serial sections with known orientation. All references to stages, cell numbers or the position of Kr^+ transcripts were confirmed in at least five different embryos and by use of the *ftz* probe in parallel sections.

ture from a highly localized pattern becomes visible when the plane of the migrating polar plate forms an angle of ~45° relative to the longitudinal axis of the embryo (Fig. 2b). Within 15 min, and in the absence of mitotic divisions, an additional narrow zone of Kr^+ gene expression occurs ventrally about four cells (equivalent to a segmental anlage) behind the Kr^+ central domain. This zone is itself four cells wide, extends over the mesoderm anlage and appears to be restricted to a ventral medial strip of ectoderm contiguous to the mesoderm anlage (Fig. 2b). This pattern becomes obscured subsequently by additional new Kr gene expression spreading posteriorly in both mesodermal and ectodermal cells. By the end of germ-band extension, at ~6 h post-fertilization, Kr^+ transcripts have accumulated from the posterior edge of the cephalic furrow throughout the thoracic and abdominal anlagen. Higher signal intensity occurs in the ventral region, the posterior edge of the cephalic furrow and especially over the adjacent thoracic and the anterior abdominal anlagen. Furthermore, variation in signal intensities follows a metameric pattern along the extended germ band (Fig. 2c).

During the extended germ-band stage, Kr transcript accumulation becomes gradually restricted to cells of the mesoderm and possibly also neuroblast cells (Fig. 2c–e). Labelling of the previous Kr^+ posterior domain becomes limited to a few cells (Fig. 2c) that form the anlage of the malpighian tubules (see refs 17, 18 for details) and persists after these organs are formed (Fig. 2d, e). Kr^+ transcripts of the previous Kr^+ anterior domain appear predominantly in a medially narrow strip of cells above

the dorsal roof of the stomodeum (Fig. 2d), which corresponds to the site of the developing oesophagus and includes the somatogastric nervous system¹⁷.

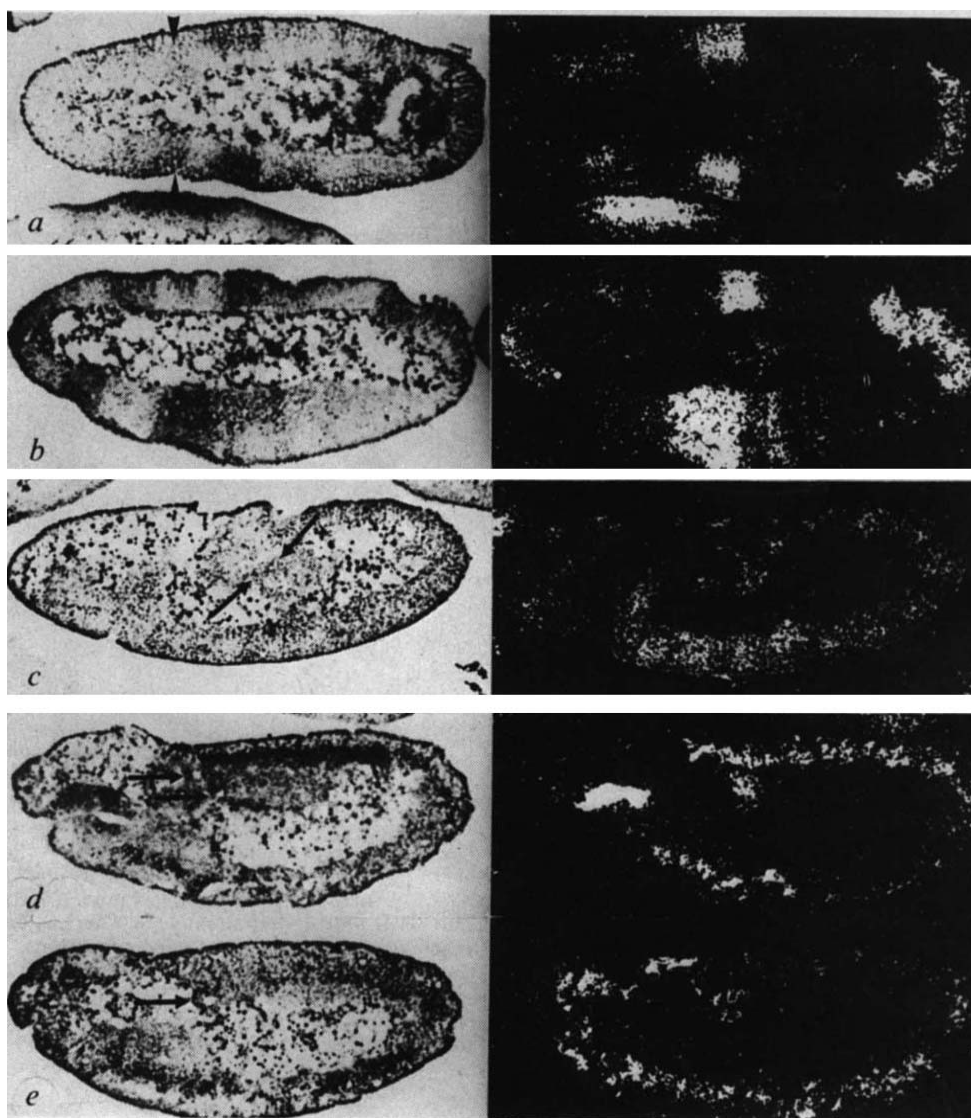
By the beginning of germ-band shortening, ~8 h post-fertilization, the spatial pattern of Kr gene expression has decreased to a low level of Kr^+ transcript accumulation (approaching background) throughout the embryo (data not shown).

Cytoplasmic Kr^+ activity

Phenotype rescue response of homozygous (amorphic) Kr^1 embryos was used for determining the localization of Kr^+ activity in different regions of wild-type embryos, and to assay the spatial requirement for Kr^+ gene product(s) in the Kr mutant embryos.

Embryos of *Ddc*ⁿ⁷ Kr^1 /SM1 parents were injected with cytoplasm of wild-type donor embryos. Homozygous Kr^1 embryos, which can be distinguished from their siblings by their *Ddc* phenotype (see refs 8, 10 for details), were scored for phenotype rescue effects. Some of these embryos developed a segment with normal polarity anterior to the sixth abdominal segment and closely resembled the intermediate, rather than the strong, Kr phenotype of uninjected Kr^1 embryos. The weak phenotype rescue effect on segmentation was only observed when the cytoplasm was taken from the middle region (30–70% egg length; posterior is 0) of blastoderm-stage embryos and only if injected into the same region of pole-cell stage Kr embryos (see Table 1). The region of highest Kr^+ activity in wild-type embryos and the rescue-responsive region in Kr embryos correspond

Fig. 2 Localization of Kr^+ transcripts in sections of embryos of early gastrula and early germ-band extension stage. Format, orientation and experimental conditions are as described in Fig. 1 except that the probe concentration was $0.25 \mu\text{g ml}^{-1}$ and the autoradiographic exposure was for 9 weeks. **a**, Frontal section deriving from the dorsal half of an early gastrula-stage embryo having just formed the cephalic furrow (arrowheads); **b**, medial section of an early gastrula embryo slightly older than the embryo shown in **a**. Note that pole cells have already migrated dorsally. **c**, Slightly tangential section of a late germ-band extension embryo. Note that label extends along the posterior edge of the cephalic furrow, that Kr^+ transcripts accumulate highest in the thorax and anterior abdominal region, that differences in intensities suggest a metamereric pattern of Kr^+ transcripts and that Kr^+ transcripts accumulate in cells forming malpighian tubules (arrow). **d**, **e**, Localization of Kr^+ transcripts in parallel, slightly oblique sagittal sections of an extended germ-band stage embryo $\sim 7\text{--}8$ h post-fertilization. Note the strong hybridization signal in the oesophageal region (somatogastric nervous system), the absence of label over ectoderm, Kr^+ transcript accumulation in mesoderm (including neural tissue) and in malpighian tubules (arrow). Note that when probe concentrations $>0.25 \mu\text{g ml}^{-1}$ were used, the Kr^+ anterior domain as well as initial signs for spreading (**a**, **b**) were not detectable above nonspecific background, a fact that we attribute to the higher signal-to-noise ratio of lower probe concentrations in *in situ* hybridizations (see ref. 27).



(within the limits of resolution) to the initial Kr^+ central domain. Malpighian tubules never formed in our experimental conditions (see Table 1).

Discussion

We report here a complex pattern of Kr gene expression that is regulated in three spatially distinct domains during early stages of embryogenesis. The pattern of Kr gene expression has no direct correspondence in the segment pattern of the strong Kr phenotypes (see below). This lack of correspondence and the complex changes in Kr gene expression (Fig. 2) were unexpected in light of earlier studies on *fushi tarazu* (*ftz*) and *engrailed* (*en*), two other genes affecting segmentation in which the periodic patterns of gene expression correspond to the affected regions of the respective mutant phenotypes¹⁹⁻²¹.

The Kr^+ central domain is $\sim 12\text{--}14$ cells wide at blastoderm stage and corresponds to the zone of highest Kr^+ activity in cytoplasm of wild-type embryos, to the rescue-responsive region in amorphic Kr embryos (Table 1), and to mesothoracic, metathoracic and the first abdominal primordia on the blastoderm fate map^{17,22}. In all but the weakest Kr phenotype^{8,10}, the gap in the segment pattern is larger than these three segments. This suggests that regions of blastoderm cells that do not express the Kr gene are affected by the absence or presence of Kr gene expression in neighbouring cells, and that the formation of segments affected in strong Kr alleles may involve activity of

other genes (see below) and/or could be mediated directly by the spread of Kr gene expression and/or Kr^+ gene product(s) during early gastrulation (Fig. 2).

A comparison of Kr and *ftz* gene expression suggests that different initiation and maintenance of gene activity exist for different classes of segmentation genes. Expression of both genes is first detected at syncytial blastoderm stage. However, *ftz*⁺ transcripts were first observed in a broad band encompassing the 15–65% region of the embryo excluding the yolk nuclei. During the following two nuclear divisions, the *ftz*⁺ transcripts gradually become restricted and localized to seven bands of cells corresponding to the anlagen of segments deleted in *ftz* mutants¹⁹.

It has been proposed¹⁰ that Kr gene expression is activated by a hypothetical gradient of positional information²³, thought to be generated by maternal-effect genes such as *bicaudal* (see ref. 10 for details). Two aspects of Kr gene expression seem to be consistent with this interpretation: the initial appearance of the Kr^+ central domain during the period of first detectable zygotic transcription^{24,25} (Fig. 1a); and the occurrence of Kr^+ transcripts in the peripheral layer of syncytial blastoderm nuclei and the central yolk nuclei of the corresponding region (Fig. 1b).

After blastoderm, significant levels of Kr^+ transcripts accumulate in the posterior abdominal anlagen. As these form normally in amorphic Kr embryos^{7,8,10}, Kr^+ activity may only be required

Table 1 Age of recipient embryos at the time and site (% egg length) of injection

Age of donor Oregon P2 embryos	Region where cytoplasmic sample was taken (% egg length)	Age of recipient embryos at the time and site of injection (% egg length)	No. of injected homozygous <i>Ddc</i> ⁿ⁷ <i>Kr</i> ¹ embryos scored	homozygous segmental rescue (%)	No. of <i>Kr</i> ¹ embryos with: malpighian tubules (%)
pc-sb	40-60	pc 0-10	52	0	0
		20-30	26	0	0
		40-60	55	0	0
		70-90	33	0	0
mb	0-10	40-60	59	0	0
	10-30	40-60	53	0	0
	30-40	40-60	52	6 (12)	0
	40-60	40-60	68	18 (26)	0
	60-70	40-60	54	5 (9)	0
	70-90	40-60	57	0	0
sb	40-60	40-60	55	0	0
eb	40-60	40-60	54	9 (16)	0
lb	40-60	40-60	53	24 (45)	0
	0-10	0-10	49	0	0
	0-10	eb 0-10	26	0	0
		mb 0-10	28	0	0
		lb 0-10	51	0	0
lb	40-60	lb 40-60	58	0	0
mb	40-60	pc 0-10	62	0	0
		20-30	52	0	0
		30-40	53	4 (8)	0
		40-60	68	18 (26)	0
		70-80	52	3 (6)	0
		80-100	56	0	0

Embryos from *Ddc*ⁿ⁷ *Kr*¹/SM1 parents were injected and subsequently handled as described elsewhere⁸. The amount of injected material was ~300 pl, which corresponds to ~3% of egg volume. Cytoplasm was taken from precisely staged donor wild-type embryos (Oregon P2); <10% of egg volume was taken and transferred into the referenced region of recipient embryos. In measuring percentage of egg length, the zero point was placed at the posterior end. *Kr*¹ homozygous embryos were scored after development to hatching larvae by their *Ddc* phenotype as described previously^{8,10}. Malpighian tubule development was scored during development and related to *Kr*¹ embryos that could be unambiguously scored later¹⁰. The development of at least one segment with normal polarity adjacent to the sixth abdominal segment was scored as 'segmental rescue'. (The criteria for this rescue response are outlined in ref. 8). pc, Pole-cell stage; sb, syncytial blastoderm stage; eb, early blastoderm stage when the oolemma just starts to engulf the nuclei; mb, mid-blastoderm stage before elongation of nuclei (see text); lb, late blastoderm stage when nuclei are engulfed by the oolemma.

anterior to the sixth abdominal segment. Injected cloned *Kr*⁺ DNA sequences⁸ or cytoplasm from a middle region of wild-type embryos (Table 1) as well as hypomorphic *Kr*⁺ activity in *trans*¹⁰ cause the formation of one or a few additional anterior abdominal segments in amorphic *Kr* embryos. This suggests that initiation and/or maintenance of these segments require a low level, and the formation of additional segments towards the mesothorax requires increasing levels of *Kr*⁺ activity that might relate to the graded levels of *Kr*⁺ transcripts at extended germ-band stage (Fig. 2c). Furthermore, the preceding spread of *Kr* gene expression (Fig. 2b) and its sharp restriction at the posterior edge of the cephalic furrow (Fig. 2c) suggest regulation through the action of and/or interaction with other zygote effect genes⁴⁻⁷. The best candidates for *Kr* interaction are the products of the other two members of the gap class of segmentation genes, *knirps* and *hunchback*, mutation of which cause the deletion of subsets of contiguous segments, partially overlapping on either side of the *Kr* domain⁷. The interaction of both gene products with *Kr* (products) might be required to initiate and/or to maintain the formation of a subset of segmental anlagen defined by the respective mutant phenotypes⁷ which become specified further by the action of other segmentation and homoeotic genes.

The *Kr*⁺ expression of the posterior domain, which appears two mitotic divisions after that in the *Kr*⁺ central domain, probably plays a part in malpighian tubule development. This can be deduced from the position of this domain on the blastoderm fate map^{17,18}, and more directly from the final restriction of *Kr*⁺ transcripts to the malpighian tubules. The involvement of *Kr* in malpighian tubule development is to be expected because amorphic *Kr* mutants lack these organs⁹. In contrast,

the localization of *Kr*⁺ transcripts after germ-band extension is surprising as related defects in *Kr* embryos have not yet been observed. Note, however, that the *Kr*⁺ locus is the source of several developmentally regulated transcripts (ref. 11 and U.B.R. *et al.*, unpublished data) sharing common sequences with the cDNA clone used in this study. One or more of these may be uniquely responsible for segmentation function, malpighian tubule development and the predicted secondary *Kr* effects that remain to be established. Further characterization and localization of sequences unique to each transcript will be required to determine the different effects of *Kr*, a segmentation gene that seems to be more complex on the molecular level than expected on the basis of genetic studies.

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LETTERS TO NATURE

Wind from a starburst galaxy nucleus

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Galaxies with highly active star formation regions (starburst galaxies) are inferred to have high supernova rates in the region of activity^{1,2}. If most of the supernova energy input is thermalized, a strong wind is driven out of the active region. The wind is probably so fast that gravitational forces are not involved. We present here an analytical solution for the wind which is driven from a region of uniform mass and energy deposition. The solution is applied to M82—the best observed case of a starburst galaxy. The model can be directly compared with the gas pressure in M82 and with a wind velocity deduced from radio spectra. The galaxy does have clouds orbiting out of the disk. The interaction of the wind with the clouds can give rise to streaming motions and to X-ray emission. The wind is expected to create a hole in any diffuse gas in which the galaxy is embedded. The wind may have a significant role in the evolution of a starburst galaxy in that it transfers gas, which is probably heavy-element enriched, from the central regions to the outer parts of the galaxy.

We assume that the wind is spherically symmetrical and that gravitational forces are negligible. The total mass and energy input are $\dot{M}$ and $\dot{E}$ respectively. The equations for the flow are³:

$$\frac{1}{r^2} \frac{d}{dr} (\rho u r^2) = q \quad (1)$$

$$\rho u \frac{du}{dr} = -\frac{dP}{dr} - \frac{q}{u} \quad (2)$$

$$\frac{1}{r^2} \frac{d}{dr} \left[\rho u r^2 \left(\frac{1}{2} u^2 + \frac{\gamma}{\gamma-1} \frac{P}{\rho} \right) \right] = Q \quad (3)$$

where for $r < R$, $q = \dot{M}/V$, $Q = \dot{E}/V$, $V = \frac{4}{3} \pi r^3$, and for $r > R$, $q = Q = 0$; u is the wind velocity, r is the radial coordinate, ρ is the density, P is the pressure, and γ is the adiabatic index. The radius R is the limit of mass and energy input. The solution is simplified by using the Mach number $M = u/c$, where $c = (\gamma P/\rho)^{1/2}$ is the sound speed, as a variable. Analysis of the solution shows that a smooth transition from subsonic flow at the centre to supersonic flow at large r requires that $M = 1$ at $r = R$. The solution is

$$\left(\frac{3\gamma+1/M^2}{1+3\gamma} \right)^{-(3\gamma+1)/(5\gamma+1)} \left(\frac{\gamma-1+2/M^2}{1+\gamma} \right)^{(\gamma+1)/[2(5\gamma+1)]} = \frac{r}{R} \quad (r < R) \quad (4)$$

and

$$M^{2/(\gamma-1)} \left(\frac{\gamma-1+2/M^2}{1+\gamma} \right)^{(\gamma+1)/[2(\gamma-1)]} = \left(\frac{r}{R} \right)^2 \quad (r > R) \quad (5)$$

The other physical variables can be found from the integrated form of equations (1) and (3). The solution for $\gamma = 5/3$ is illustrated in Fig. 1 and is given in various limits in Table 1.

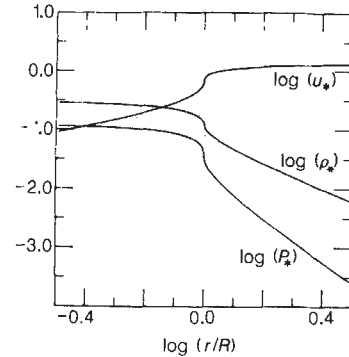


Fig. 1 The wind solution as a function of r/R , where R is the radius of the region of mass production $\dot{M}$ and energy production $\dot{E}$. The dimensionless variables are $u^* = u/(\dot{M}^{-1/2} \dot{E}^{1/2})$, $\rho^* = \rho/(\dot{M}^{3/2} \dot{E}^{-1/2} R^{-2})$, and $P^* = P/(\dot{M}^{1/2} \dot{E}^{1/2} R^{-2})$. For $|log(r/R)| > 0.5$, the solution is described by the expressions in Table 1.

Rieke *et al.*¹ estimate a supernova frequency in the central region of M82 of 3 per 10 yr. An independent estimate is available from the rate of decline of compact radio sources which are presumed to be the remnants of supernovae⁴; this leads to one supernova per 3-5 yr. If the energy of each supernova is 10^{51} erg, the energy production rate is $\sim 10^{43}$ erg s⁻¹. We assume a fraction α of this energy is deposited in a low-density gas which fills most of the volume. The factor α is to account for radiative losses by dense gas and inaccuracies in the supernova rate.

With the above supernova rate, the mass input from supernovae is $\sim 1 M_\odot$ yr⁻¹ and we take $\dot{M} = \beta M_\odot$ yr⁻¹. The factor β is to account for possible cooling of the supernova ejecta (for example, if it is clumped as in Cas A) and other uncertainties. The compact radio sources observed by Kronberg *et al.*⁵ occupy a region about 600×100 pc in projection. The fact that the energy input region is flattened reduces the accuracy of the model in describing the region, but we estimate that this introduces an error of at most a factor of 2 or 3 in the physical quantities (see the dependences on R in Table 1). We take $R = 200$ pc.

The input parameters for M82 are thus $\dot{E} = 10^{43} \alpha$ erg s⁻¹, $\dot{M} = \beta M_\odot$ yr⁻¹ and $R = 200$ pc. These lead to a central pressure and density of $P_0 = 7.8 \times 10^{-9} \alpha^{1/2} \beta^{1/2}$ dyn cm⁻² and $\rho_0 = 1.2 \times 10^{-25} \alpha^{-1/2} \beta^{3/2}$ g cm⁻³, and a velocity at $r = R$ of $u = 2.8 \times 10^3 (\alpha/\beta)^{1/2}$ km s⁻¹. An estimate of the wind velocity at $r \approx R$ is suggested by the steepening of the radio spectral index away from the centre; Seaquist *et al.*⁶ deduce a wind velocity of $\sim 2,000$ km s⁻¹. While this applied to relativistic electrons, in our model they would be expected to move with the gas. This result implies $\alpha \approx \beta$. As the escape velocity from the galaxy is

Table 1 Properties of the wind solution for $\gamma = 5/3$

	$r \ll R$	$r = R$	$r \gg R$
$\rho/\dot{M}^{3/2} \dot{E}^{-1/2} R^{-2}$	0.296	0.113	$0.0563 (r/R)^{-2}$
$P/\dot{M}^{1/2} \dot{E}^{1/2} R^{-2}$	0.118	0.0338	$0.0106 (r/R)^{-10/3}$
$u/\dot{M}^{-1/2} \dot{E}^{1/2}$	$0.269 (r/R)$	0.707	1.414

a few hundred km s⁻¹, the neglect of gravitational terms in equations (1)–(3) is justified.

The gas pressure in M82 can be estimated from observations of relatively dense gas at temperature $T \approx 10^4$ K. From the [S II] doublet ratio, O'Connell and Mangano⁷ obtained electron densities $n_e = 1,800$ cm⁻³ in central H II regions, 800 cm⁻³ at 12 arc sec S (180 pc), and ≤ 100 cm⁻³ at 36 arc sec S (540 pc). In terms of pressure, this corresponds to $P_0 = 5 \times 10^{-9}$ dyn cm⁻², in fair agreement with α and β both ~ 1 . The decrease in pressure is $P/P_0 = 0.44$ at $r \approx R$ and $P/P_0 \leq 0.05$ at $r \approx 3R$. If the dense ionized gas is exposed to the wind, its pressure is determined by the total ram pressure, $P + \rho v^2$, in the wind, but if it is shielded from the direct wind interaction, its pressure is determined by the thermal pressure in the wind. In the model, $P/P_0 = (1, 0.29, 0.0025, 8 \times 10^{-7})$ and $(P + \rho v^2)/(P + \rho v^2)_0 = (1, 0.76, 0.10, 0.01)$ for $r/R = (0, 1, 3, 10)$. The observed radial dependence is slightly steeper than the ram pressure case. Young H II regions can have overpressures compared with the surrounding medium, so other estimates of n_e are desirable. Houck *et al.*⁸ have deduced $n_e = 120 (+280, -120)$ cm⁻³ from a S III line ratio in the central part of M82. Rodriguez and Chaisson⁹ analyse the radio recombination lines and free-free emission and deduce that the emitting gas has $n_e = 300$ cm⁻³ with a filling factor of 0.04. This analysis requires several assumptions. If $n_e \approx 300$ cm⁻³ at the centre, then $(\alpha\beta)^{1/2} \approx 0.01$ or $\alpha \approx \beta \approx 0.1$. In another analysis of radio recombination line and free-free emission, Seaquist *et al.*⁶ deduced that the emitting gas has $n_e = 30$ cm⁻³ and a filling factor of unity. This interpretation is inconsistent with the presence of a thermally driven wind. A high pressure is indicated by the nonthermal radio emission from the central region, which implies a minimum energy density in relativistic gas of 3×10^{-9} erg cm⁻³ (ref. 10).

M82 is a source of extended X-ray emission^{5,11}. Watson *et al.*¹¹ suggest one possibility for the emission: gas with $n = 0.2$ cm⁻³ and $T = 10^7$ K filling 0.1 of a volume with radius of about 1.7 kpc. In the model, the gas density falls too rapidly for the wind to produce the emission itself. However, there are clouds orbiting M82 (ref. 7). If some of this gas has a density 0.1 cm⁻³, it can be shocked to a temperature $T \approx 10^7$ K. The pressure in the gas should be equal to the total wind ram pressure. With the above parameters $P = 5.5 \times 10^{-10}$ dyn cm⁻², which is 0.07 of P_0 in the model. This value of the ram pressure is reached at about $4R$ (800 pc) in the model. While the model can perhaps reproduce the X-ray properties with $\alpha = \beta = 1$, the case $\alpha = \beta = 0.1$ is too weak in X-ray emission. This indicates a high central pressure. The explanation of the X rays as resulting from a wind-cloud interaction is consistent with the observed correlation of X-ray emission with optical filaments¹¹.

The outflowing wind can drive clouds out from the centre of the galaxy. From ref. 12, the terminal velocity reached by a cloud in the constant velocity part of the flow is $v_t = 430 \alpha^{1/4} \beta^{1/4} (r_0/1 \text{ kpc})^{-1/2} \sigma_{-3}^{-1/2}$ km s⁻¹ where r_0 is the initial distance of the cloud from the centre of the galaxy and σ_{-3} is the mass column density of the cloud in units of 10^{-3} g cm⁻². A standard cloud in our Galaxy has $\sigma_{-3} \approx 1$. Clouds which are substantially affected by the wind have $r_0/v_t \leq \tau$, where τ is the age of the wind. With $\tau = 3 \times 10^7$ yr and $\alpha = \beta = 1$, this requires $(r_0/1 \text{ kpc})^{3/2} \sigma_{-3}^{1/2} \leq 13$. Thus, standard clouds can be directly accelerated and instabilities induced by the wind-cloud interaction may lead to further outflow. This motion, in combination with the orbital motions of clouds, may contribute to the complex velocities observed in M82 (refs 13, 14). The gas responsible for the X-ray emission is subject to outward acceleration, so it must be continually produced by the break-up of clouds.

While high-column-density clouds are not much affected by the wind, diffuse gas is swept away. From ref. 15, the extent of the wind is $14(\alpha\tau_7^3/\rho_{-27})^{0.2}$ kpc where τ_7 is the age of the wind in units of 10^7 yr and ρ_{-27} is the density of ambient gas in units of 10^{-27} g cm⁻³. At this radius, a fast shock wave is driven into the medium. Cottrell¹⁶ has noted a diminution of the H I surface density along the northern axis of M82. This may be related to the sweeping action of the wind. The central supernovae are

expected to mix heavy elements into the hot gas, so the wind should enrich the surrounding medium with metals.

We have applied our model to the galaxy M82 because this galaxy has been the subject of many observational investigations. However, the model should apply to any galaxy where there is a region of concentrated energy input which is capable of driving a fast wind. As in M82, extended X-ray emission is expected from the galaxy, particularly if there are clouds in the wind. The major prediction of the model is the variation of thermal pressure and ram pressure with radius. The magnitude of the pressures is related to the mass and energy input. Various observed parameters can be related to the pressures. The model cannot be expected to give detailed agreement with observations because it does assume spherical symmetry. An improved model would take into account an asymmetrical region of mass and energy input and an asymmetrical environment (for example, a galactic disk) into which the wind is expanding.

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First measurements of thermospheric winds in Antarctica by an optical ground-based method

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The installation of a Fabry–Perot interferometer at Halley (75.5° S, 26.8° W; $L = 4.2$), Antarctica, has now allowed the first comparison to be made between Southern Hemisphere ground-based thermospheric wind measurements and the predictions of a three-dimensional time-dependent thermospheric global circulation model. Because some terms of the thermospheric momentum equation are more dependent on geomagnetic than geographic latitude, the large separation of these that exists at Halley provides a distinct contrast to the dynamic behaviour observed in the more frequently studied northern polar thermosphere. Although the initial results from the experiment are in general agreement with the model, some consistent and significant differences between the observed wind field and that predicted are evident in the morning sector. These may be related to uncertainties in mapping magnetospheric boundaries to ionospheric heights in the Southern Hemisphere.

Because of the high geographic latitude of Halley, there is sufficient darkness, near winter solstice, to allow continuous observations of atmospheric optical emissions. Halley, with an invariant latitude of 60.8° S, normally lies in the sub-auroral zone and hence the major contributor to the sky brightness at the 630-nm line is the diffuse airglow of low intensity, many orders of magnitude below the visual threshold. Auroral emissions occur spasmodically, providing a much brighter, and often sharply spatially structured, contribution.

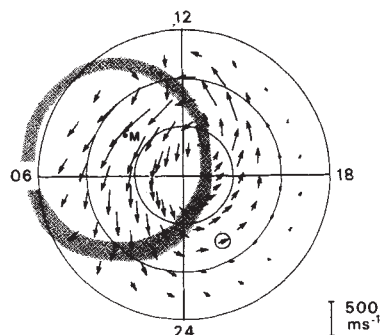


Fig. 1 Model simulation of the Southern Hemisphere wind field at 240 km altitude, 0000 UT. Latitudinal range is 60° S to the pole (geographic). The region of strong anti-sunward flow is displaced from the geographic pole towards the geomagnetic pole (labelled M), away from the location of Halley (circled vector). The position of the auroral oval is indicated by the shaded region.

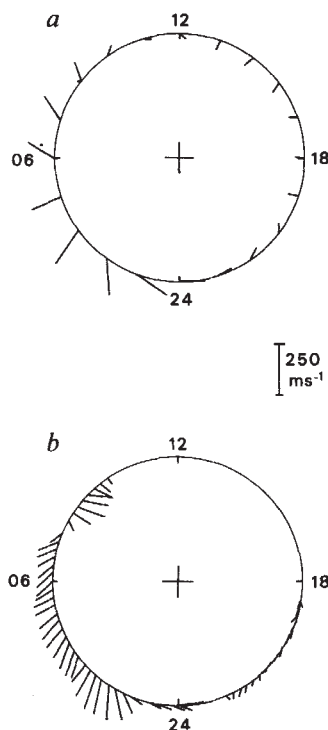


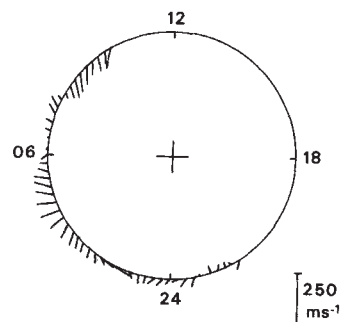
Fig. 2 a, Model simulation of winds over a 24-h period for Halley. Wind vectors are shown at hourly intervals in UT. The solar and magnetospheric parameters used in the model are chosen to represent the conditions prevailing during the selected observation period. b, Neutral wind data from 12 to 13 July 1983 (1830–0945 UT). Vectors interpolated at 15-min intervals are shown, their tails lying on a circle describing the locus of Halley through a 24-h period.

The instrument¹ used in this study has a large aperture (150 mm) Fabry-Perot etalon using zerodur spacers, 10 mm in length. The circular fringe patterns are recorded using an imaging photon detector, and the data are stored on magnetic disks capable of holding the observations made over several days. A scanning mirror system allows measurements to be made in the four geographical cardinal directions at 30° elevation, and also in the zenith.

In the present investigation, the Doppler shift of the 630 nm $O(^1D)$ emission is used to measure the bulk motion of neutral constituents in the F-region of the ionosphere. At the latitude of Halley, the peak in the volume emission rate occurs at ~240 km. Satellite data², rocket measurements³ and theoretical calculations⁴ have shown little altitudinal structure in the wind field over the region of maximum intensity (due to the high kinematic viscosity) and so the observations are representative of the bulk neutral motion at this height. From a continuous set of observations, a time series of line-of-sight velocities is calculated in each of the cardinal directions. Using the assumptions outlined by Smith⁵, for example, a negligible vertical velocity in the wind field, the line-of-sight components are converted into a vector representing net horizontal flow.

In the Southern Hemisphere, the large separation between the geographic and geomagnetic poles causes a considerable longitudinal modulation of the thermospheric wind in a solar time/geographic latitude coordinate system. This is referred to as the Universal Time (UT) effect^{4,6}. The rotation of the magnetic pole about the geographic pole, in a Sun-Earth reference frame,

Fig. 3 Neutral wind data from 15 to 16 July (2145–1000 UT). The same reversal feature can be seen at about 0800 UT on this geomagnetically quiet day (K_p average 1+).



carries with it a region of strong anti-sunward flow^{7,8}. Because Halley is almost diametrically opposite to the magnetic pole in geographic coordinates, the station will always sample an area that is on the edge of, or well separated from, the fast streaming region, whereas a station at a latitude similar to that of Halley but located on the opposite side of the geographic pole would observe quite different diurnal wind variations. The general circulation pattern and the centering of the high-velocity region on the magnetic pole can be seen clearly in the three-dimensional time-dependent model of Fuller-Rowell and Rees⁷ shown in Fig. 1.

The auroral oval is a circular region of visual arcs displaced 3° from the geomagnetic pole, towards the dark hemisphere⁹ (Fig. 1). Its boundary becomes closest to Halley at about 0200–0300 UT, resulting in an increased auroral contribution in the measurements. Conversely, at 1400 UT the auroral oval is very distant, that is, close to the geographic pole, and the sky illumination from the 630-nm line is then only due to airglow.

Model calculations for the Southern Hemisphere predict a relatively weak wind field at Halley, attributable to its low geomagnetic latitude (65.8° S). For the period from 1000 to 2000 UT, in a simulation of moderately disturbed conditions (Fig. 2a), the predicted velocities remain below ~80 m s⁻¹. Depending on the time of day, these winds are driven to a different extent by ion drag resulting from the sunward ion convection at sub-auroral latitudes, and by the pressure-gradient force resulting from the diurnal heating cycle due to solar extreme ultraviolet plus any local heating effects. The zonal component is very small during this period, there being a fairly consistent poleward velocity.

The period of lowest velocity predicted by the model occurs in the pre-midnight sector, around 2000 UT, when the Halley ionosphere is in the weak sunward ion convection equatorward of the auroral-zone boundary. The sunward ion drag is opposed and nearly balanced by the anti-sunward force resulting from the global diurnal pressure gradient; this causes a region of near stagnation in the wind field. The highest velocities are expected in the period 0300–0700 UT when the station is close to the magnetic noon-midnight meridian downstream from the geomagnetic pole. These mainly equatorward winds are driven by the diurnal pressure-gradient force and ion drag from the high-speed cross-polar ion convection, acting in a similar direction. Joule heating due to ionospheric currents probably also contributes to these winds. During this period, Halley is downwind of the area of highest velocities in the polar cap.

Comparison between the modelled (Fig. 2a) and measured (Fig. 2b) wind vectors for 12–13 July 1983 shows that there is good agreement for some periods of UT, such as 0100–0500 UT, but poor agreement at others (0700–0900 UT). The ionospheric and magnetospheric inputs to the model have been selected to be representative of the moderate geomagnetic activity sustained throughout this day, when the K_p index¹⁴ varied from 3+ to 5 and the Halley fluxgate magnetometer records showed prolonged moderate activity. The model includes a polar convection electric field with a total cross-cap potential of 70 kV. High-latitude heating and ionization effects resulting from auroral electrons are also included^{10,11}.

For the observed winds, which are interpolated at 15-min intervals, the errors in determining the velocity depend on the

intensity of the 630-nm emission. In the local-time evening sector ($LT = UT - 2$), the observed winds are weak and the intensities are low. Thus, the estimated error in velocity is up to $\pm 40 \text{ m s}^{-1}$. Directional information is, therefore, not reliable, although there are indications of a weak equatorward wind, the zonal component changing from eastwards to westwards near 2300 UT. With greater intensities present through the rest of the night, the typical error is $\pm 20 \text{ m s}^{-1}$.

The most significant difference is that the observed wind between 0700 and 0900 UT is mainly poleward and of moderate strength, in this case, $\sim 100 \text{ m s}^{-1}$, whereas the model predicts a weak equatorward flow. Modifications arising from the orientation of the interplanetary magnetic field y -component (B_y) were considered; however, model simulations representing both B_y positive and negative conditions predicted an equatorward velocity until around 1100 UT. There are several mechanisms that might contribute to this discrepancy; for example, time-dependent changes of geomagnetic activity generate complex direct changes of thermospheric winds in the polar regions. However, this suggestion is not supported by the observation of a similar feature on other, rather quiet days (see Fig. 3). The most likely explanation of this lies in the modelling difficulty of mapping magnetospherically controlled phenomena, such as the convection electric field and the particle precipitation boundaries, accurately in the Southern Hemisphere. Currently, the model uses the invariant latitude offset of 16° at the South Pole and 11° in the north. The latter gives an excellent prediction of the location of geomagnetically controlled phenomena in the Northern Hemisphere^{7,8,12}. In the Southern Hemisphere,

however, a large separation exists between geographic and dip latitude frames. Because of asymmetries in the field, this offset is approximately 29° at Halley (dip latitude = 46.4° S). Arguments have been presented that the dip latitude reference frame might be more appropriate when it is related to dynamic processes such as the effects of winds and electric fields¹³. A further development of this comparison could be that the topological mapping of features of the precipitation boundaries and convection patterns within the southern polar region do not make a simple one-to-one correspondence with those in the Northern Hemisphere, where a far greater body of ground-based and rocket-borne data have been available to complement spaceborne observations.

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Imaging of atomic clouds outside the surfaces of gold crystals by electron microscopy

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The structure of small metal crystals has been intensively examined by high-resolution electron microscopy (HREM). In particular, multiply-twinned gold and silver crystals have been characterized using the profile-imaging method^{1,2} at atomic resolution, and reconstructed metal surfaces observed². Crystal structure images of large gold clusters consisting of 55 gold atoms arranged in a cubeoctahedron have been recorded³ using $2.5 \text{ \AA}$ resolution, and crystal growth, row by row, on a $\{111\}$ surface has been documented⁴ using a low-light-level silicon-intensified target television (TV) camera and video system with an on-line image processor. Direct imaging of rearrangements of atomic columns on extended gold surface⁵ established that profile imaging can provide information about surface self-diffusion. The motion of surface atoms, recorded with a real-time video tape-recorder (VTR) system, and the formation of surface atom steps on $\{100\}$ surfaces, although not $\{111\}$, has also been reported recently⁶. Dynamic HREM observations at TV rate showing defect motion in gold⁷ and CdTe (refs. 8, 9) has given information on 'in-lattice' rearrangements of columns of atoms. We report here surface profile images recorded with the electron beam along the $\langle 110 \rangle$ direction with spatial resolution of $\sim 2.0 \text{ \AA}$ which reveal changes in occupancy of the atom columns often within periods of less than 0.1 s. Surfaces with several adjacent atom columns involved in rapid structural changes frequently interacted with a cloud of atoms extending out to $9 \text{ \AA}$ off the crystal and changes in shape and density of the clouds were recorded. Although these clouds have already been described^{6,10}, the present work is the first to analyse these events properly and to describe them in detail. The motion of atomic columns and the existence of atom clouds revealed here may have important consequences for crystal growth, surface science and catalysis studies.

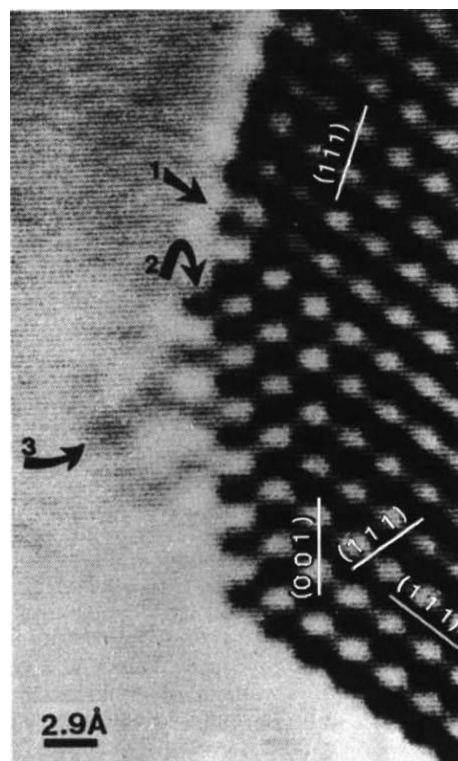


Fig. 1. Image with a gold surface profile recorded along $[1\bar{1}0]$ showing a cloud of gold atoms outside a (001) surface. Arrow 1 marks a twin plane (111) in the crystal. A column consisting of a maximum of seven gold atoms at the (001) surface is marked 2 and the outer limit of the gold atom cloud is marked 3. The fine horizontal lines in the image are scan lines of the TV monitor. Time of registration, 0.13 s.

Gold clusters containing 55 atoms, $\text{Au}_{55}[\text{P}(\text{C}_6\text{H}_5)_3]_{12}\text{Cl}_6$, were made by reducing $(\text{C}_6\text{H}_5)_3\text{PAuCl}$ with B_2H_6 in benzene¹¹ and a drop of the sample, dispersed in methanol, was then placed on an amorphous holey carbon film. The clusters were observed to be randomly oriented and spread across the entire film. The

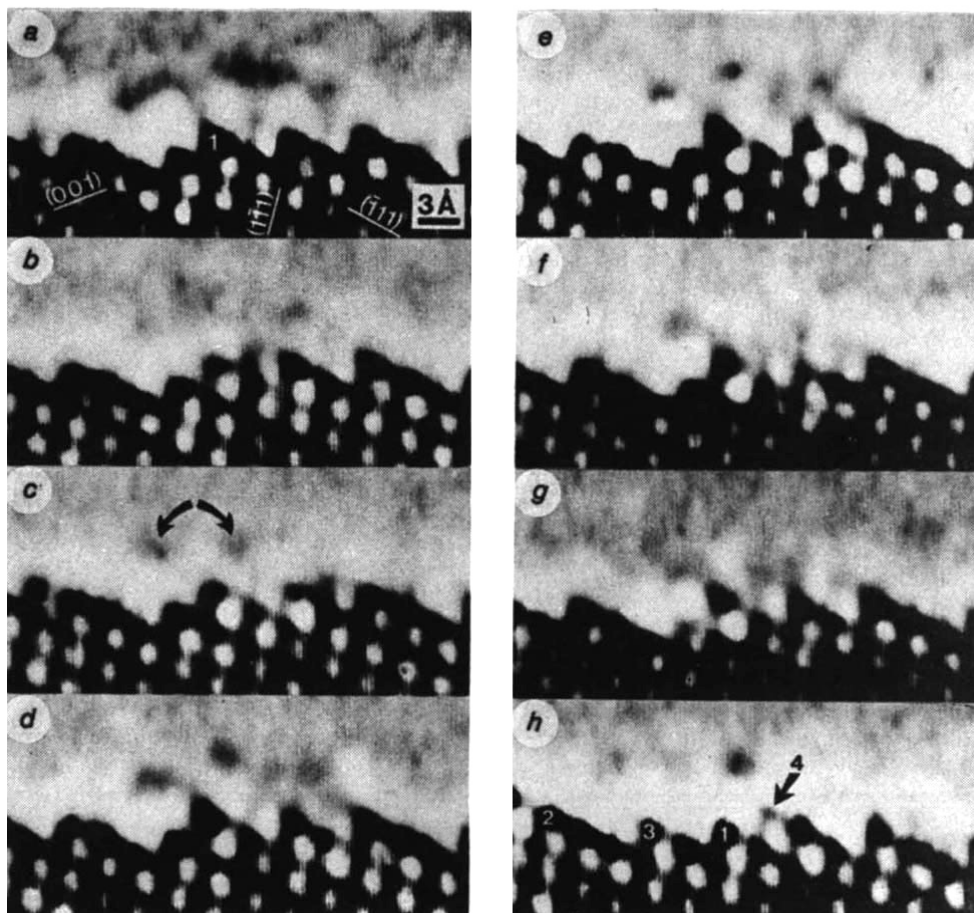


Fig. 2 Sequence of images showing surface profiles of a rough $(\bar{3}31)$ gold surface, built up by small steps at $(\bar{1}11)$, $(1\bar{1}1)$ and (001) surfaces, all recorded within a few seconds and each with an exposure time of 0.13 s. The images show how the cloud of atoms outside the surface changes shape and interact with some gold columns. The column marked 1 is changing position during the interaction. Columns 2, 3 and 4 are new in *h*. See text for explanation.

organic ligands and chlorine atoms were removed by the vacuum system from the area irradiated by the electron beam. The remaining crystal with the 55 Au atoms was unstable and started to grow at the expense of other crystals⁴. The surfaces of the growing crystals were very clean and usually did not show any contamination with amorphous structure during the growth process. Moreover, because of the high intensity of the electron beam in normal viewing conditions ($\sim 20\text{--}30\text{ A cm}^{-2}$), it seems highly unlikely that any chlorine (or phosphorus) atoms from the original clusters could remain attached to these crystals for any extended period. It would also be difficult to explain why atoms other than gold could sit in exact register with the bulk lattice, as has always been observed here.

The microscope used for this study was a JEM-4000EX in the basic ultra-high-resolution top-entry configuration. The electron accelerations voltage was 350 kV and the spherical aberration coefficient of the objective lens was 1.0 mm. The point resolution is better than $2\text{ \AA}$ at 350 kV, making it possible to resolve the distances ($2.5\text{ \AA}$) between the gold atom columns in the $\langle 110 \rangle$ projection. Fibre-optically coupled TV system, Gatan 622, having a 0.15-mm yttrium-aluminium-garnet screen, was used for the video-recording but without an image intensifier. The images recorded on video tape were passed through a Quantex DS-30 digital video processor for noise reduction by an exponentially weighted sliding-average method with an averaging time constant of $4/30\text{ s}$ (four frames) using $512 \times 512 \times 256$ resolution. It was possible to 'freeze' the image at any time with the use of the frame-grabber facilities in order to take photographs of the TV monitor. The normal electron optical image magnification of the microscope was $\times 600,000$ and the final magnification on the TV monitor was $\sim 20 \times 10^6$. The photographs of the video monitor generally showed very little interference from the scan lines of the monitor, since the distances of interest in the gold crystals were of the order of 10 times the distance ($0.3\text{ \AA}$) between the scan lines.

The observations of atomic rearrangements in real time can

be divided into three types: small crystals with sizes generally $\leq 30\text{--}40\text{ \AA}$ in diameter change their crystal shape and orientation continuously^{4,6,10} and often very quickly; larger crystals show localized column hopping, sometimes within $1/30$ th of a second¹²; and outside some specific surfaces, clouds of atoms in motion can be seen. It is significant that such clouds have so far not been seen adjacent to $\{111\}$ surfaces but were very common outside $\{100\}$ surfaces, as also reported by Iijima⁶, and outside $\{110\}$ and $\{331\}$ surfaces, which has not been reported previously. This characteristic of the atom clouds is seen in the central (001) surface profile image of an Au crystal shown in Fig. 1, which was recorded with the electron beam parallel to the $[1\bar{1}0]$ direction of the crystal. Note that the adjacent surfaces are both $\{111\}$. The cloud of atoms is localized only outside the (001) surface and shows some sort of regularity almost in periodicity with the crystal lattice. The outermost layer of atoms belonging to the (001) surface consists of only partly filled columns (one marked with arrow 2, Fig. 1) during the time (0.13 s) of registration. This crystal originated from a cube-octahedral crystal of 55 gold atoms, with a square-shaped (001) surface. If its shape is assumed to be basically the same during crystal growth, then the depth of the present (001) surface profile is seven gold atoms. The column marked 2 has a contrast almost as dark as the neighbouring inner columns. Assuming that there are five or six gold atoms in this column during the time of registration, some steps in the projected crystal surface, hardly showing any contrast at all, consist of only one or two atoms. The centre of the dark contrast of columns of this layer seems to be in periodicity with the crystal lattice. However, this is not the case for the next layer. Two such columns can be recognized. The distances between the columns of the $\{111\}$ planes are $2.5\text{ \AA}$ in this projection but the outer two are $\sim 2.8\text{ \AA}$ out from the regular lattice. The contrast of the two columns is more diffuse and this is even more pronounced for the column (arrow 3, Fig. 1) of the outer visible cloud, which is $7.5\text{ \AA}$ out from the outermost regular (001) lattice plane.

In most crystals studied so far, the clouds of gold atoms range out to 7–9 Å from the outermost crystal lattice plane and it is interesting to conjecture whether this distance is significant for {100} surfaces of gold. This possibility will only be answered by further investigations of other metals.

The continuous changes to the shapes of the clouds, as well as the column hopping, have been well documented for many crystals on our video tapes. It has been found that columns of atoms can be out of their normal lattice positions for a short time while in interaction with the cloud of atoms. Such behaviour is shown in Fig. 2. This sequence of images of the same crystal profile, assumed to be projected along $[1\bar{1}0]$, represent the ever-changing life of a $\{331\}$ surface over a few seconds' time span. The images reveal that this $\{331\}$ surface is not an even 3×1 -stepped surface on the atomic scale, but rather is composed of atom steps with different lengths of (001), $(\bar{1}11)$ and $(1\bar{1}1)$ surfaces. Thus, a more proper name here would be 'piecewise $\{331\}$ '.

The atom column marked 1 in Fig. 2a is of major interest in this sequence. In this figure, three columns are interacting with parts of the cloud looking like tornadoes. The shapes of the cloud clearly show the pathways for the gold atoms on their way out of or into the three columns of the lattice. The next image (Fig. 2b), recorded less than a second later, shows how the contrast of column 1 is weaker, indicating that some atoms must have moved out to the cloud. Just to the right of column 1 is some off-lattice contrast, most likely as a result of the transport of atoms from the middle 'tornado' shown in Fig. 2a. Column 1 has slightly changed its lattice position; the distance to the neighbouring column to its right is $\sim 10\%$ longer than normal. That distance has further increased in the next image (Fig. 2c), recorded less than a second later.

Figure 2c–g correspond to changes happening in real time within less than a second. The cloud shown in image c seems to have two positions (marked with arrows) with darker contrast. Their distances from column 1 are ~ 4.8 Å, separated by an angle of 80° . The distance between the two dark positions is ~ 6 Å. Column 1 has moved to a position out of lattice 3.6 Å from its bottom-right neighbour while the distances to the columns immediately below and below to the left are close to normal, 2.5 Å. Note that one outer column two steps to the right of column 1 has a weaker contrast and an off-lattice position just to its right similar to the position of column 1 in image b. Images d and e show how the contrast of the two darker positions in the cloud has increased and that there is an interaction with column 1 which has become darker than in image c. This implies a transport of atoms from the cloud to column 1. The two dark positions at the cloud are 5.8 Å apart in e and the angle mentioned earlier has decreased to 69° . The darker right contrast is 5.1 Å from column 1 and this contrast disappears in the next image, f. There is obviously a strong interaction between the cloud and the columns to the right of column 1 in images d–g. The position of column 1 seems to be the same in images c–f. Careful comparison of images c–g shows that there is a transport of atoms to column 1 in the beginning ((c)–(e)) and then an emigration out to the cloud in f and g. In image g, the neighbouring column below and to the left has lost atoms to such an extent that column 1 has less 'bonding' in this direction. The distance to its neighbours has then increased to 3.6 Å in the bottom and bottom-left directions. The column two steps to the right of column 1 has darker contrast and thus has more atoms in its row. A few seconds later (image h), column 1 has gone back to its 'normal' lattice site while three new columns (marked 2, 3 and 4) have been added to the surface. Column 4 is still very weak in contrast and is presumably only a few atoms deep. The cloud outside the $\{331\}$ surface has one major concentration of contrast about 4.5 Å from column 4.

It is interesting that most dynamical events occur where the normal sequence of the steps in the surface is disturbed. It is also important to appreciate that, because of the continuous atomic rearrangements, these images of atom clouds actually represent some sort of time and spatial average of atomic column

positions. The non-uniform background visible, for example, in Fig. 2 is due solely to this variation. Note also that the observed atomic motion is caused, in part, by the incident beam, as defocusing of the beam leads to reduced activity. However, surface self-diffusion is known to occur in face-centered-cubic metals even at room temperature¹³. It would be interesting, and relevant to studies of real catalysts, to repeat the types of observations reported here, once some sort of specimen heating cartridge becomes available which is compatible with atomic resolution.

This work has demonstrated that off-lattice dynamical events outside crystal surfaces can be recorded at atomic resolution. In small particles of cubic-close-packed gold, the interaction between {100} and {331} surfaces and clouds of atoms has been found to be very rapid. It may well be that column hopping and changes of cloud shape are an indication of how atoms locate the most favourable lattice position during crystal growth. The phenomena described here also suggest that it should be possible to monitor the way in which other atoms interact with a metal surface, and this should be of fundamental importance for studying heterogeneous catalysis.

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Dissolved zinc in rivers

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Reliable measurements of the concentrations of dissolved trace metals in rivers are needed for calculating oceanic chemical mass balances, understanding continental weathering and freshwater chemistry, and evaluating anthropogenic chemical perturbations. For dissolved zinc, it has been suggested¹ that typical river concentrations are as high as 450 nmol kg^{-1} . However, it has also been proposed² that most published values are incorrect and the true value of dissolved zinc may be 2 orders of magnitude lower. Because zinc is one of the most commonly used metals in the industrial world, the sampling and analysis of this element in natural waters is particularly prone to contamination². We present here the dissolved zinc concentrations from various rivers draining both pristine and industrially influenced environments. These samples were collected and analysed by methods that have yielded reliable oceanic data for other metals³. The data indicate that in relatively undisturbed systems dissolved zinc concentrations are typically only 10^{-9} – $10^{-8} \text{ mol kg}^{-1}$, with some dependence of concentration on pH. In industrially influenced river systems, however, zinc concentrations can be 1–2 orders of magnitude higher.

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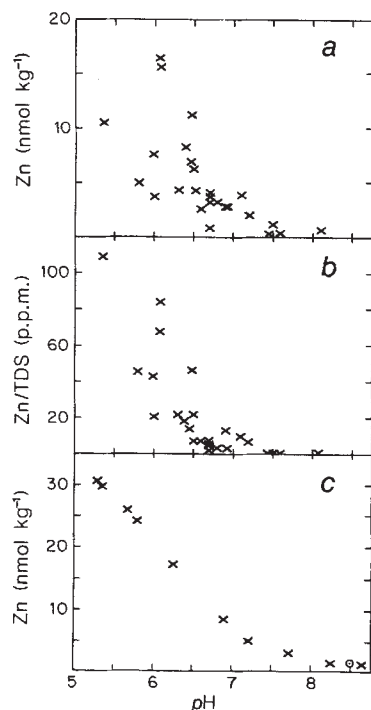


Fig. 1 Dissolved zinc plotted against pH. *a*, Zinc in relatively undisturbed major rivers including the Yangtze (Chiang Jiang) and tributaries of the Amazon and Orinoco. *b*, Zinc normalized to total dissolved solids for the same set of major rivers. *c*, Zinc in pH-adjusted aliquots of Mississippi River water (April 1984, 103 mg l⁻¹ suspended load, pH 7.7); the adjusted aliquots were allowed to equilibrate overnight before filtration and analysis. Precision of these zinc data is estimated to be 10%; at pH~4.5 the dithiocarbamate method recovered ~92% of the zinc.

Samples were obtained using acid-cleaned linear polyethylene bottles. The bottles were normally attached to a Plexiglas holder at the end of a long non-metallic pole and immersed below the river surface. Replicate samples were obtained when possible. Sampling sites were chosen to avoid nearby tributaries and any immediate sources of contamination. After collection, samples were filtered (0.4 µm Nuclepore) and acidified to pH < 2 with purified hydrochloric acid. In cases where a comparison was made, no difference was noted between samples filtered immediately on collection and those stored in the dark and filtered several days later.

Dissolved zinc was analysed by graphite furnace atomic absorption spectrometry using a Perkin-Elmer 5000/HGA 400 combination. Most samples were first preconcentrated by the cobalt-pyrrolidine dithiocarbamate method³ with the coprecipitation carried out at pH ~ 4.5. Samples with high zinc concentrations (> 15 nmol kg⁻¹), however, were determined directly.

Table 1 lists dissolved zinc concentrations for various major rivers draining generally undeveloped regions. In Fig. 1*a*, a relationship between dissolved zinc and pH is evident for these rivers. A similar trend has been observed for beryllium⁴. This relationship could result from variations in: (1) the amount of zinc in different source rocks; (2) the leaching of trace elements in different weathering regimes; or (3) the distribution of zinc between the adsorbed and dissolved phases.

Several lines of evidence suggest that it is the adsorption behaviour of zinc that is primarily reflected in Fig. 1*a*. In Fig. 1*b*, zinc concentrations have been normalized to total dissolved solids (TDS) to give a crude indication of the solubilization of zinc relative to the major components of the weathered source rocks. The values in Fig. 1*b* may be compared with average zinc

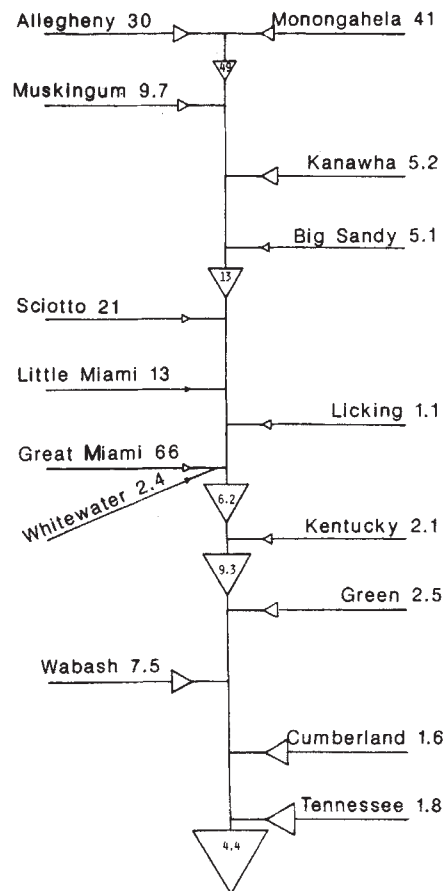


Fig. 2 Dissolved zinc in rivers of the Ohio Valley. Area of the triangle is proportional to the mean flow of the tributary. These tributaries account for about three-quarters of the mean flow of the Ohio River. Northern tributaries are to the left; southern tributaries are to the right.

concentrations in several rock types⁵: granite, 52 p.p.m.; shale, 120 p.p.m.; limestone, 20 p.p.m.; basalt, 100 p.p.m. Although acidic rivers have zinc/TDS ratios similar to rock concentrations, alkaline rivers are markedly depleted in dissolved zinc. This suggests that the zinc/pH relationship may be chemical in nature rather than being related to source rock concentrations. Further evidence of a chemical relationship was found in an experiment in which the pH of aliquots of unfiltered Mississippi River water was adjusted with small amounts of clean acid or base. The resulting dissolved zinc/pH curve, shown in Fig. 1*c*, is similar to isotherms for the adsorption of zinc on various natural and metal oxide surfaces⁶, though some dissolution of surface coatings may also be occurring. A similar experiment in which an aliquot of unfiltered river water was adjusted to pH 3, allowed to equilibrate and then readjusted to an alkaline pH (open circle, Fig. 1*c*) demonstrated that the process is reversible. A final experiment, in which aliquots of unfiltered Mississippi River water were spiked with small quantities of dissolved zinc, allowed the calculation of a distribution coefficient for zinc of ~10⁵ ((mol Zn adsorbed per kg solid) per (mol Zn dissolved per l of solution)). This coefficient indicates that the Mississippi sample had 10 times more adsorbed than dissolved zinc.

Close inspection of the Amazon Basin data confirms the importance of adsorption in controlling fluvial zinc concentrations. Using tributary flow estimates⁷ to calculate the flow-weighted sum of dissolved zinc for the basin yields a concentration of 6.4 nmol kg⁻¹. This is significantly higher than the actual dissolved zinc concentration near the mouth of the Amazon. Similar non-conservative behaviour has been observed for copper⁸. The discrepancy in this case results primarily from the Rio Negro, an acidic tributary with low suspended load that con-

Table 1 Dissolved zinc in rivers draining relatively undisturbed areas

River	Date	pH	Zn (nmol kg ⁻¹)
Amazon Basin			
Amazon (Iquitos)	July 1976	7.50	1.2
Napo	July 1976	6.70	2.6, 4.0?
Javari	July 1976	6.45	6.9, 10.6?
Içá	June 1976	5.99	7.6, 12?
Jutaí	June 1976	6.07	17, 19
Juruá	June 1976	6.80	3.2, 5.5?
Japura	June 1976	6.52	4.3, 4.3
Tefé (Tefé)	June 1976	6.08	16
Solimões (Laranjari)	June 1976	6.93	2.8
Coari (Coari)	June 1976	6.48	11, 12
Purus	June 1976	6.39	8.1, 8.4
Negro (Manaus)	June 1976	5.36	10.4, 10.6
Madeira	June 1976	6.70	3.9, 4.4
Trombetas (Oriximiná)	June 1976	6.50	6.1, 6.4
Tapajós	June 1976	6.91	2.8
Xingu	June 1976	7.1	3.9
Amazon (Mouth at high flow)	June 1976	6.7	3.8
Amazon (Mouth at low flow)	December 1982	7.45	0.3, 0.3
Orinoco Basin			
Ventuari	June 1982	6.0	3.7
Atabapo	June 1982	4.3	5.0
Inírida	June 1982	5.2	27
Guaviare	June 1982	6.6	2.6
Meta	June 1982	6.7	0.9
Apure	June 1982	7.6	0.3
Caura	June 1982	6.3	4.1, 4.5
Orinoco (Puerto Ordaz)	June 1982	7.2	2.0
Caroní	June 1982	5.8	5.0
Yangtze River (Chiang Jiang)			
Yangtze (Mouth at high flow)	June 1980	8.1	0.6, 1.0
Yangtze (Mouth at low flow)	November 1981		1.2, 1.2

tributes ~25% of the Amazon's flow^{7,9}. In the flow-weighted sum, the Rio Negro furnishes ~40% of the total zinc. However, when the waters of the Rio Negro mix with the more alkaline and suspended sediment rich main channel waters, much of the zinc contributed by the Rio Negro must be removed from solution by adsorption. Other factors may also be important in determining the dissolved/adsorbed distribution of zinc in rivers, including organic matter concentrations and the physical/chemical makeup of the suspended load. Our observations also indicate that alkaline rivers have low dissolved zinc because they have higher suspended loads than acidic rivers.

That samples from different rivers fit into a consistent and interpretable pattern is evidence for the validity of this data set. However, some rivers have been found to have zinc concentrations higher than expected from Fig. 1 (Table 2). As sampling methods were the same for these rivers and replicate samples were generally in agreement, we believe that the high concentrations did not result from contamination through improper sample handling. Examination of dissolved zinc concentrations in the Ohio River and its tributaries (Fig. 2) does, however, reveal some interesting trends. Zinc in the main channel of the Ohio has its highest concentration upstream and lowest downstream. This is reasonable because the two furthest upstream tributaries have very high zinc concentrations whereas the largest downstream tributaries have low dissolved zinc. Additionally, note that most of the tributaries to the south of the main channel have dissolved zinc concentrations <6 nmol kg⁻¹ whereas most of the northern tributaries are >6 nmol kg⁻¹. As the states to the north of the Ohio River (Ohio and Indiana) have twice the population density of the states to the south (West Virginia, Kentucky and Tennessee)¹⁰, it seems plausible that this pattern of higher than expected zinc concentrations results from the addition of zinc from anthropogenic sources. The Whitewater, which has the lowest zinc of the northern tributaries, is also the only northern tributary without a major population centre in its basin. Tributaries with dissolved zinc concentrations >10 nmol kg⁻¹ include the Allegheny and Monongahela, which receive contributions from the Pittsburgh metropolitan area and the coal mining regions of western Pennsylvania, and the three small rivers draining the population centres of south-central Ohio

Table 2 Dissolved zinc in rivers of the US

River	Date	pH	Zn (nmol kg ⁻¹)
Ohio Valley			
Allegheny (Pittsburgh, Pennsylvania)	May 1984	6.86	29, 31
Monongahela (Pittsburgh, Pennsylvania)	May 1984	7.22	39, 44
Ohio (Wheeling, West Virginia)	May 1984	7.34	49, 49
Muskingum (Zanesville, Ohio)	May 1984	7.55	16, 19?
Muskingum (Marietta, Ohio)	May 1984	7.66	9.6, 9.7
Kanawha (Winfield, West Virginia)	May 1984	7.4	5.1, 5.4
Big Sandy (Louisia, Kentucky)	May 1984	7.15	5.1, 5.1
Ohio (Greenup Dam)	May 1984	7.42	12, 14
Scioto (Portsmouth, Ohio)	May 1984	7.87	20, 22
Little Miami (Milford, Ohio)	May 1984	8.1	13, 16?
Licking (Alexandria, Kentucky)	May 1984	7.63	1.1
Great Miami (Cleveland, Ohio)	May 1984	8.0	65, 66
Whitewater (Elizabethtown, Ohio)	May 1984	7.95	2.4
Ohio (Warsaw, Kentucky)	May 1984	7.45	6.0, 6.4
Kentucky (Lockport, Kentucky)	May 1984	7.28	1.9, 2.3?
Ohio (Cannelton Dam)	May 1984	7.27	9.3
Green (Beach Grove, Kentucky)	May 1984	7.32	2.5, 2.5
Wabash (New Harmony, Indiana)	May 1984	8.1	7.5
Cumberland (Barkley Dam)	May 1984	7.44	1.6, 1.6
Tennessee (Kentucky Dam)	May 1984	7.10	1.8, 1.8
Ohio (Mound City, Illinois)	May 1984	7.49	4.4, 4.5
Mississippi River			
Mississippi (Cape Girardeau, Missouri)	May 1984	7.70	2.9, 3.5?
Mississippi (Baton Rouge, Louisiana)	September 1983	8.1	1.6, 1.8
Mississippi (Baton Rouge, Louisiana)	April 1984	7.72	2.8, 2.9
Atchafalaya (Krotz Springs, Louisiana)	April 1984	7.6	2.8
Other US rivers			
Connecticut (Old Saybrook, Connecticut)	April 1983	7.1	14, 16
Mullica	August 1983	5.81	39, 40
Merrimack (West Newbury, Massachusetts)	February 1983	6.73	200, 200
Vermilion (Lafayette, Louisiana)	April 1984	7.1	3.8, 4.1
Delaware (West Trenton, New Jersey)	April 1984	7.38	60, 61
Delaware (Philadelphia, Pennsylvania)	April 1984	7.05	200, 240?
Schuylkill (Philadelphia, Pennsylvania)	April 1984	7.58	70, 75
Susquehanna (Holtwood, Pennsylvania)	April 1984	7.54	12, 13
Potomac (Great Falls, Maryland)	April 1984	7.75	8.3, 8.4

(Scioto-Columbus; Little Miami-eastern Cincinnati; Great Miami-Dayton).

This survey of zinc in rivers allows the estimation of the fluvial dissolved zinc flux to the oceans. The average river bicarbonate concentration of 850 μ M (ref. 1) indicates an alkaline pH. The major rivers in Table 1 which discharge directly into the ocean are all alkaline. The flow-weighted concentration of these major rivers suggests a dissolved zinc concentration from natural sources of ~3 nmol kg⁻¹ for the world average. Anthropogenic contributions are difficult to assess. If we assume that half of the runoff from North America and Europe (~10% of the world river flow) is affected by anthropogenic sources, then these rivers would have to have zinc concentrations of ~30 nmol kg⁻¹ to double the world average. The data in Table 2 suggest that a doubling of the world average is probably an upper limit, although European rivers may have substantially more anthropogenic zinc than US rivers^{11,12}. Additional modification of the zinc flux may occur in estuaries where dissolved concentrations can be altered by desorption from the suspended load, flocculation of organic matter and sedimentary diagenesis. Our preliminary results from several estuaries (Mississippi, Amazon, Connecticut) indicate that desorption and sedimentary inputs may increase the dissolved flux, but probably by less than

a factor of two. In heavily polluted estuaries, zinc removal has occasionally been observed^{11,13}. From this information we estimate the present-day fluvial dissolved zinc flux to be $2 \pm 1 \times 10^8$ mol yr⁻¹.

These river data suggest an oceanic residence time for dissolved zinc of ~50,000 yr. This is calculated from the pre-anthropogenic river concentration with a 50% estuarine increase and assuming a deep ocean zinc concentration of 6 nmol kg⁻¹ (ref. 14). The residence time will be shorter if other natural sources of zinc to the ocean—including atmospheric and hydrothermal inputs and diagenetic release of zinc from coastal sediments—are of importance.

The results presented here demonstrate that levels of dissolved zinc in rivers are lower than was previously recognized. Additionally, dissolved zinc concentrations are dependent on pH. This suggests that acidification of natural waters by acid rain, mine drainage, or waste disposal may result in greatly increased levels of dissolved zinc.

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Micrometre-sized volcanic glasses in polar ices and snows

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Explosive volcanic eruptions can follow long cycles of activity¹, and the material that they inject into the atmosphere may affect the climate². We report here the discovery of ultrathin micrometre-sized glass shards from such eruptions, extracted from both ancient Antarctic ices and recent snow samples from Greenland, in which they have been well preserved by 'deep freezing'. When such shards have been preselected using a high-voltage electron microscope, microanalysis of their eight major constituent elements gives new clues about the origin of major volcanic acid fallout previously detected in the cores (including important characteristics of their parent eruptions), and reveals complex volcanic ash layers that could reflect periods of world-wide enhanced volcanicity. Moreover, the very small Reynolds numbers of the micrometre-sized shards make them promising tracers of dust transport processes in the ancient atmosphere.

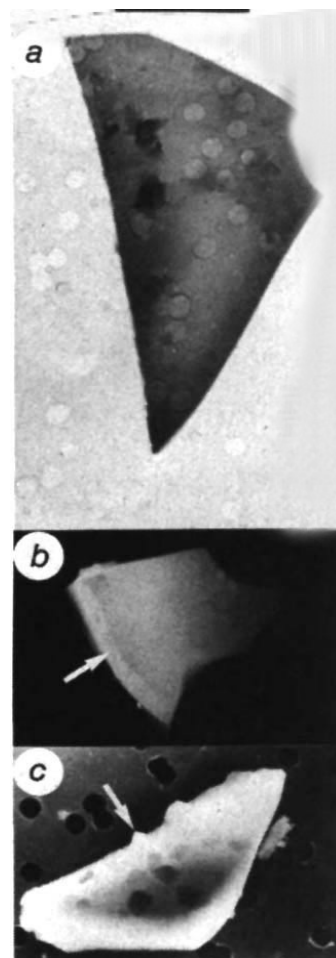


Fig. 1 Transmission micrographs (scale bar, 2.5 µm) taken by C. Jouret with the HVEM, that runs at 2–3 millions volts. These micrographs illustrate both the ultrathin texture of the glass shards found in polar ice and snow, and the holey electron microscope membranes, directly used for filtering melted ice in the ultra-clean facilities at Grenoble (see the tiny holes of the membranes in the upper and lower micrographs). *a*, A grain from the Tambora acid ice level in Antarctica; the clear image of the membrane holes through this grain reflects its ultrathin (~2,000 Å-thick) and homogeneous texture. *b*, A ~4,000-Å thick and curved flake (see the thin cross-section under the white arrow) from the El Chichón fallout in recent Greenland snow. *c*, A shard extracted from the ~6,000-yr old volcanic layer in the Dome-C core in Antarctica, containing three distinct populations of glasses; this type of inhomogeneous grain which is coated with a spectacular 'micro-grained' deposit composed of insoluble crystals as indicated by the white arrow, was not retained for accurate chemical analyses.

Volcanic fallout of sulphuric acid has now been detected in both Arctic³ and Antarctic^{4,5} ice cores through measurement of peaks in the ice acidity. These measurements, as pioneered by Hammer³, yield accurate depths of major volcanic layers along a core, but they cannot be used to infer characteristics of their parent eruptions. Thus there have been a few attempts to identify, in these acid fallout layers, tephra from non-Antarctic explosive eruptions^{6,7}, but these analyses, are not conclusive, and Kyle *et al.*⁷ who undertook microanalyses of grains extracted from Antarctic cores, stated that 'to date only tephra from Antarctic volcanoes have been identified'.

We have collected micrometre-sized glass shards from distant volcanic eruptions in ice cores and snow samples found in central regions of Antarctica and Greenland, respectively. For this work, we first fabricated a new type of holey electron microscope membrane, composed of a ~200-Å thick carbon replica of a Nuclepore filter, with an optimum pore diameter

Table 1 Analyses of micrometre-sized volcanic glass shards

Oxides	El Chichón					Taupo			Tambora		
	Tephra	Glass	Strato	Snow		Tephra	Ice		Tephra	Ice	
SiO ₂	70.02 (0.57)	69.44 (1.12)	69.44 (1.30)	69.17 (0.50)		76.15 (0.66)	66.54 (1.51)		57.85 (0.81)	59.45 (1.56)	
TiO ₂	0.30 (0.06)	0.30 (0.10)	0.34 (0.08)	0.38 (0.19)		0.32 (0.12)	0.64 (0.14)		0.56 (0.05)	0.66 (0.22)	
Al ₂ O ₃	17.25 (0.40)	17.58 (0.46)	17.87 (0.56)	17.13 (0.23)		13.39 (0.30)	15.77 (0.50)		19.92 (0.23)	16.42 (0.49)	
FeO*	1.25 (0.18)	1.41 (0.28)	1.40 (0.37)	1.89 (0.52)		1.84 (0.29)	6.27 (0.97)		4.08 (0.52)	8.99 (0.69)	
MgO	0.14 (0.09)	0.36 (0.20)	0.35 (0.13)	0.38 (0.13)		0.17 (0.07)	1.51 (0.21)		1.51 (0.19)	2.82 (0.70)	
CaO	1.89 (0.18)	1.83 (0.26)	1.89 (0.28)	1.80 (0.23)		1.51 (0.13)	4.45 (0.36)		3.15 (0.25)	5.43 (0.78)	
Na ₂ O	4.00 (0.28)	4.08 (0.87)	3.90 (0.81)	4.48 (0.67)		3.67 (0.48)	3.96 (0.92)		6.80 (0.48)	4.14 (0.49)	
K ₂ O	5.09 (0.23)	4.97 (0.55)	4.72 (0.38)	4.75 (0.34)		2.93 (0.20)	0.82 (0.09)		6.10 (0.18)	2.04 (0.38)	
†	31	19	15	6		11	24		11	13	

Concentration (in wt%) of eight major oxides in micrometre-sized volcanic glass shards extracted from different samples. In each column, we give the mean and s.e.m. of the chemical analyses. Samples from the El Chichón eruption include: pumice and dust grains with sizes of $\sim 100 \mu\text{m}$ (column 2) as well as microglasses with sizes of $\sim 10 \mu\text{m}$ (column 3) collected near the volcano; stratospheric dust grains (column 4) collected by the B-57 aircraft of NASA in May 1982 just after the eruption; dust grains extracted from snow samples collected at the Dye 3 station in central Greenland (column 5), and in which we discovered a short-lived glass fallout from this eruption occurring as early as June 1982. Samples from other volcanic fallout include: tephra from the 186 Taupo eruption (column 6) and grains from the Taupo ice section in the Dome-C core (column 7); tephra from the 1,815 Tambora eruption (column 8) and grains from the Tambora ice section (column 9) in the Dome-C core. The useful chemical microanalysis of these micrometre-sized glasses requires their careful preselection with a HVEM. A simple SEM+EDAX observation is not sufficient: First, the observation with the SEM of a shard surface morphology, typical of glass, can be completely misleading. Indeed the habit of crushed grains from both crystalline minerals and organic material with imperfect cleavages frequently exhibits a shard morphology; Second, inclusions within the grains and/or secondary particles easily collected underneath them during the filtration of melted ice on a Nuclepore filter (which will be subsequently analysed with the EDAX system if the shards are thin enough) cannot be detected during a SEM observation, to reject such chemically inhomogeneous aggregates for the EDAX analysis.

* All Fe calculated as FeO.

† Number of analysis in mean.

of $\sim 0.4 \mu\text{m}$ (see the holes in Fig. 1a). These membranes were fixed on numbered gold grids and then laid on top of a Nuclepore filter to filter directly $\sim 50 \text{ g}$ of melted ice. The grains were next coated with a 200-Å thick carbon film, thus being firmly encapsulated into a carbon microcrucible 'drilled' with $0.4\text{-}\mu\text{m}$ holes on one side. This procedure markedly reduced both grain losses and beam degradation effects during analysis.

Strict criteria are necessary for identifying and selecting appropriate volcanic glasses with sizes of a few micrometres. Previous attempts to identify such tiny glasses by relying only on a scanning electron microscope (SEM) equipped with an X-ray energy dispersive spectrometer (EDAX system) were unreliable (see Table 1). We first used a high-voltage transmission electron microscope (HVEM), to select truly amorphous grains with an homogeneous structure free of inclusions and/or secondary particles accreted on both the top and bottom surfaces of the grains. Only such preselected areas of a grain can be usefully analysed with the SEM+EDAX system. We could thus identify real glasses exhibiting the characteristic spectra of eight major constituent elements (Si, Ti, Al, Fe, Mg, Ca, Na and K) expected in volcanic glasses, and reject up to 80% of amorphous grains of organic and/or biogenic origin.

We analysed 30 distinct sections of three ice cores, drilled at the Dome C station in central Antarctica over the period 1978–80 (ref. 8), with only two sections found at depths of ~ 12 and 104 m showing strong acidity peaks. These are attributed on the basis of a $\sim 5\%$ accurate dating of the cores, respectively to the 1,815 Tambora and the 186 Taupo cataclysmic eruptions. We also analysed 18 successive sections of a 1-m thick slab of recent snow, collected by C. Hammer at the Dye 3 station in central Greenland in June 1983, and in which we searched for the micrometre-sized glasses of the 1,982 El Chichón eruption.

Our preliminary results can be summarized as follows:

(1) Discovery of micrometre-sized volcanic glasses in the Dome-C cores. The background concentration of glassy grains in 27 Antarctic ice sections with a normal acidity is very small ($\leq 0.2\%$ of the total number of grains). But in the two sections showing strong acidity peaks, the proportion of glassy grains increased by a factor of > 50 above the background values. We also found a high concentration of glass in a $\sim 6,000\text{-yr-old}$ ice section for which no acidity measurement was available, but in which A. Gaudichet (personal communication) found a few glassy grains during a mineralogical investigation. This last observation sug-

gests that the micrometre-sized glasses could delineate volcanic fallout undetectable by other means, as a consequence of a low gas-to-dust ratio during the parent eruption.

(2) The ultrathin texture of the glass shards. Most of the polar glasses, such as those reported in Fig. 1, are ultrathin shards with thicknesses of $\sim 0.5 \mu\text{m}$. This was first shown by B. Doyle, who shot a microbeam of $\sim 5 \text{ MeV}$ α -particles at one glassy grain preselected by us, and showing a large apparent size of $\sim 10 \mu\text{m}$. The Rutherford back-scattering spectra of the incident ions clearly showed the characteristic peak expected for a very thin target ($< 1 \mu\text{m}$ thick). This 'lamella' structure was subsequently observed for even smaller grains with an improved tilting stage of the HVEM, that allows visualization of the thin edges of the grains (see Fig. 1b). From these favourable 'aerodynamical' shapes J. Taillet computed very small Reynolds numbers ($\ll 1$) for the micrometre-sized shards. Consequently, the Stokes formulae developed for laminar flow (see ref. 9) are applicable for evaluating all their aerodynamical parameters in the atmosphere. Such glass shards should thus be reliable tracers of the motion of air masses in the ancient atmosphere, giving a new mode of investigating them.

(3) Search for possible chemical differentiation of volcanic glasses deposited in polar ices and snows. It is important to know whether the micrometre-sized polar glasses show any unexpected chemical differentiation compared with ordinary tephra collected near their parent eruptions. In Table 1 (columns 2–5) we summarize a few results indicating that the micrometre-sized glasses from the cataclysmic 1,982 El Chichón eruption, found in recent snow samples from Greenland, did not show any measurable fractionation with respect to the other glasses, collected either in various types of tephra (such as pumice, dust) or in the stratosphere.

(4) Chemical composition and origin of polar glass fallout. The chemical compositions of the various products ejected through time in a given volcanic zone are quite variable. However, they are located within diagrams of geochemical evolution (see Fig. 2) which are typical of a given volcanic region. Such diagrams have already been documented for both the Taupo¹⁰ and the Tambora¹¹ volcanic zones. We analysed micrometre-sized glasses extracted either from the Taupo and the Tambora ice levels, or from dust samples of their postulated parent eruptions (see Table 1). We observed that the glasses found in both ice levels fell far outside the corresponding geochemical diagrams.

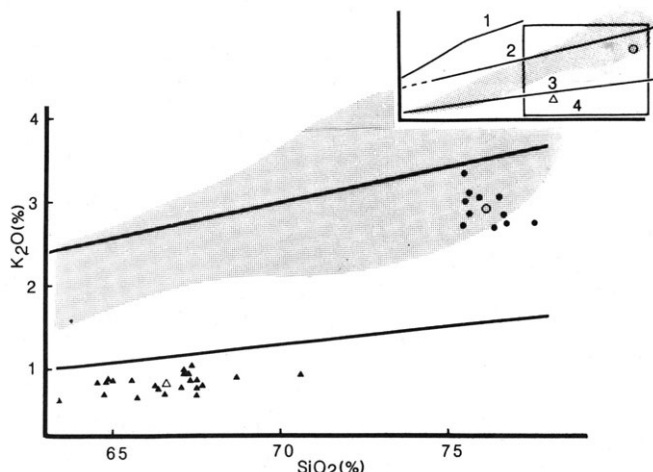


Fig. 2 Peccerillo and Taylor diagram¹³, giving the relationship between SiO_2 and K_2O contents for ordinary products of the Taupo volcanic zone. The shadowed area corresponds to 256 distinct analyses of whole rock samples quoted by Cole¹⁰. ●, and ▲, refer to our own microanalysis of 'clean-and-homogeneous' micrometre-sized glasses extracted either from dust samples of the 186 eruption, or from the Taupo acid level in the Antarctic core, respectively (○, and △, give the mean value of the corresponding analyses). The inset represents a reduction of this plot to display the localization of the four volcanic series associated with subduction volcanism. These series are delineated by the three typological boundaries lines defined by Peccerillo and Taylor, namely shoshonitic (1), high-K-calc-alkaline (2), calc-alkaline (3) and tholeiitic (4) (on the main diagram only lines (2) and (3) have been reported). For these analyses we had to learn both to minimize alkali losses during the HVEM and the SEM/EDAX analysis, and to take into consideration the very poorly defined 'shape factors' of the micrometre-sized grains, which complicate the chemical interpretation of the X-ray spectra¹⁴. For this purpose, we use a series of reference glasses with SiO_2 contents roughly similar to those of the micrometre-sized volcanic shards. We then compare the composition of the eight major elements for both a thick polished section and micrometre-sized crushed shards from a given chunk of reference glass, by measuring the mean value as well as the variance of the analyses. We first noted that in normal operating conditions of the EDAX system, the thick sample yielded a maximum count rate $\sim 1,200 \text{ s}^{-1}$, whereas the values measured for the shards could decrease to the X-ray background values ($\sim 150 \text{ s}^{-1}$). The mean values of the compositions were similar for both types of samples, when the count rate of the micrometre-sized grains was $\geq 300 \text{ s}^{-1}$ (this corresponds to a 'minimum' thickness of a few thousand Å), but a much higher variance for the analysis was deduced for the shards (see numbers within parentheses in Table 1). However, it was possible to improve this variance markedly by searching for the optimum orientation of the shards which are rather 'flat' with regard to the X-ray detector, and this was obtained when the background spectra of both types of samples were similar. (See refs 12, 15 for details of calibration experiments.)

This is clearly illustrated for the Taupo eruption in Fig. 2. We deduce from this that the glass compositions measured for these two specific layers, cannot derive from the postulated eruptions. They cannot originate either from local Antarctic eruptions. Indeed, these peculiar polar glass compositions (andesitic and dacitic poor in potassium, respectively) are both typical of a subduction volcanism. They are also very different from the sodium-rich compositions found for volcanic products from Deception Island. As discussed elsewhere¹² both constraints exclude a local Antarctic origin for these two glass fall-out; they would then constitute the first report ever made for the deposition in central Antarctica of tephra of distant ($\geq 6,000 \text{ km}$) explosive eruptions.

(5) Chemical composition and multipurpose 'stratigraphical' markers. So far, the frequency distribution of the SiO_2 concentrations measured in individual micrometre-sized glass shards from a given ice section constitutes a highly specific signature of the

parent volcanic fall-out, which could then be used as a good stratigraphical marker, to establish, for example, the occurrence of a given volcanic fallout at distant drilling sites. This should help us first in obtaining valuable 'relative' datings which could be used to severely constrain ice flow models, thus improving the dates inferred from them. One could also identify the most violent ancient cataclysmic eruptions which have affected all of the Earth by searching for their glass fallout in central regions of both Greenland and Antarctica. Moreover they give a new index of 'multiplicity' of sources for a given volcanic fallout. For example, the Tambora and the Taupo ice levels each yield a single peak distribution on the SiO_2 frequency plot indicative of a single parent eruption. In contrast the $\sim 6,000$ -yr-old level reveals three distinct peaks, associated with very different families of glass compositions, which could reflect a rapid succession of eruptions from distant volcanoes, which could, in turn, be more effective for triggering climate variations than a single eruption.

Our preliminary results indicate that the well preserved ultra-thin shards of micrometre-sized volcanic glass found in polar ices and snows are promising tracers for investigating a range of topics, such as the past activity of explosive volcanic eruptions and, possibly, changes in dust transport processes effective in the ancient terrestrial atmosphere. We stress that these studies require the careful preselection of the glass shards with an HVEM before analysing them with a SEM/EDAX system.

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Formation of secondary porosity in sandstones by quartz framework grain dissolution

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An understanding of the nature and origin of porosity in sandstones is crucial to the evaluation of their potential as hydrocarbon reservoirs. Primary porosity is normally destroyed by cementation, compaction and pressure solution during burial, but it has recently been recognized that significant secondary porosity can develop at depth¹⁻³. This discovery has been called "the most significant advance in the study of clastic diagenesis in the past decade"⁴. Several genetic types of secondary porosity have been identified; these include porosity formed by: (1) fracturing; (2) shrinkage;

(3) dissolution of sedimentary grains and matrix; (4) dissolution of authigenic cements; and (5) dissolution of authigenic replacive minerals⁵. Formation of secondary porosity by framework grain dissolution has been thought mainly to involve decomposition of feldspars and lithic grains^{6,7}. We demonstrate here that significant secondary porosity can also develop by dissolution of quartz framework grains if the rocks are exposed to alkaline pore fluids during deep burial and/or uplift.

Rotliegend gas-bearing aeolian sandstones in the Southern Permian Basin of the North Sea consist largely of quartz (>65%) with varying amounts of feldspars, lithic grains and authigenic cements. In parts of the basin where the sandstones have been buried to a depth of <3 km, feldspars, or their kaolinized remains, are relatively common, but where the rocks have been more deeply buried feldspars are virtually absent and kaolinite has largely been replaced by illite⁸⁻¹⁰. Authigenic illite cement is well-developed throughout. Many parts of the Rotliegend aeolian sequence are also cemented to varying degrees by ferroan dolomite, ankerite, siderite, anhydrite, baryte and halite.

We have recently examined several Upper Rotliegend aeolian dune sandstones from the southern North Sea using back-scattered scanning electron microscopy and energy-dispersive X-ray microanalysis. The samples described were collected from present burial depths >3.5 km, but it is likely that the maximum burial depth exceeded 4.2 km before the onset of uplift during the late Cretaceous⁹. The nature of the carbonate and evaporite cements in these rocks will be described in detail elsewhere¹¹. We point out here that significant secondary porosity can result from dissolution of quartz framework grains during burial diagenesis.

Over-sized pores are visible optically in thin sections of the sandstones studied. Shape and textural criteria suggest that some owe their origin to dissolution of early cements which partly replaced detrital constituents¹¹, but several lines of evidence indicate that many mouldic pores formed as a result of quartz framework grain dissolution during late diagenesis. First, all stages in the dissolution of quartz grains can be observed. In the early stages small (1–5 μm), irregularly-shaped voids appear within quartz grains (such as grain B in Fig. 1). These gradually coalesce to form larger voids and detached quartz relicts (such as grain A in Fig. 1). In the later stages of dissolution the grain is reduced to a ragged, highly porous aggregate. Finally, a mould is produced (Fig. 2). In some cases, the moulds have subsequently been partly infilled by authigenic illite and quartz. Second, the mouldic pores are surrounded by pellicules of authigenic illite similar to that found around the remaining quartz framework grains. Formation of the mouldic pores, therefore, post-dates this phase of illite formation (indicated by other textural evidence to be relatively early diagenetic¹¹). Third, most of the mouldic pores have not been destroyed or deformed by compaction, suggesting that they were formed during, or later than, the period of maximum burial. Fourth, mouldic pores are sometimes surrounded, but rarely infilled, by late diagenetic ferroan carbonate and/or evaporite cements. Small amounts of baryte, anhydrite and halite occasionally occur within the secondary mouldic pores, but generally only very late-stage illite and authigenic quartz are found. These textural relationships suggest that the main phase of quartz framework grain dissolution occurred during and shortly after precipitation of the ferroan dolomite, siderite and evaporite cements, around the time of maximum burial.

Some quartz grain types in the Rotliegend are evidently more susceptible to solution than others. Polycrystalline quartz, strained monocrystalline quartz, and grains containing a large number of inclusions or microcracks are generally more affected than unstrained monocrystalline quartz grains. Authigenic quartz overgrowths seem to be unaffected. These observations are consistent with previous findings concerning the relative stabilities of different quartz types^{12,13} and the importance of microfractures in controlling chemical alteration of quartz^{14,15}.

Laboratory experiments¹⁶ have shown that the solubility of quartz is low (6 p.p.m., parts per 10⁶) at a pH of <8 and 25 °C,



Fig. 1 Back-scattered scanning electron micrograph of a polished section of Rotliegend aeolian dune sandstone, showing the early stages of quartz framework grain dissolution (grains A and B). Also shown are anhydrite (1) and illite (2) pore filling cements. Pore-lining illite forms a rim on detrital quartz grains. Scale bar, 200 μm .

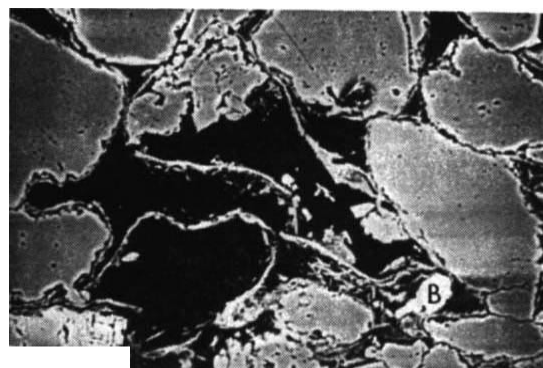


Fig. 2 Back-scattered scanning electron micrograph showing a more advanced stage of quartz framework grain dissolution. The outlines of the secondary mouldic pores are preserved by authigenic illite which has resisted dissolution. Grain B is baryte. Scale bar, 200 μm .

but rises rapidly in the pH range 8.5–10. The solubility also increases markedly with temperature, reaching ~70 p.p.m. at 100 °C and 300 p.p.m. at 200 °C (ref. 17). We conclude that quartz framework grain dissolution in the Rotliegend rocks studied took place in warm, reducing, alkaline pore fluid conditions. These pore fluids were also responsible for precipitation of late-stage ferroan carbonate and evaporite cements. The greater abundance of such cements close to faults, which acted as conduits for fluid flow, suggests that the Rotliegend sandstones may have been invaded by warm alkaline brines derived from juxtaposed Zechstein sediments at the onset of uplift in the Cretaceous^{8,9}. However, Fuchtbauer¹⁸ has shown that a time sequence of cements characterized by increasing solubility is commonly developed in sandstones, reflecting increasing ionic strength of the interstitial waters due to intrinsic diagenetic processes. The possibility that significant secondary porosity due to quartz framework grain dissolution exists in other deeply buried sandstones which contain evaporite cements, or which are associated with bedded evaporites, warrants investigation.

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The Oaxaca, Mexico, earthquake of 1931: lithospheric normal faulting in the subducted Cocos plate

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A study of the great earthquake in Oaxaca, Mexico, on 15 January 1931 shows that it was a normal fault earthquake striking east-west. The lack of evidence of surface faulting near the source region¹ and the focal depth of ~40 km inferred from body-wave modelling suggest that the earthquake occurred within the subducted slab. The large size of the earthquake ($M_s = 8.0$) strongly suggests that the rupture broke through the entire lithosphere of the subducted Cocos plate. Other large, normal fault earthquakes in the Japanese², Aleutian³, Peruvian⁴ and Sumba⁵ arcs show lithospheric faulting seawards of the trench or very close to it. The 1931 event, however, occurred farther down-dip in the slab (~150 km landward of the trench), below the bottom of what appears to be the seismogenic plate interface. We speculate here that large, although infrequent, normal fault earthquakes such as the 1931 Oaxaca earthquake may have a role in decoupling at depth the subducted slab from the upper part of the slab and perhaps from the overriding Mexican continental plate. The epicentral location of the 1858 Michoacan earthquake⁶ ($M_s \approx 7.5$) suggests that it may represent another example of lithospheric normal faulting in the Middle American arc. Such earthquakes, for which the periods of recurrence are unknown, constitute an extremely high seismic risk to population centres of Mexico.

Large ($M_s \geq 7.0$) shallow earthquakes that have occurred along the Mexican subduction zone since the installation of the World-Wide Standard Seismograph Network (WWSSN) in 1962

have been well studied⁷⁻¹¹. These earthquakes are thrust events occurring on a shallow dipping plane to the north-east (10-15°), and represent the relative motion between the subducting Cocos plate and the overriding North American plate (Fig. 1). Because all recent large shallow earthquakes are thrust type, it has been assumed that all shallow earthquakes along and near the Pacific coast of Mexico have a similar focal mechanism^{6,12}. The fault plane solution of the 1931 Oaxaca earthquake, however, shows that it had a normal fault mechanism (Fig. 2).

The polarity of the first motions of the 1931 Oaxaca event were verified by comparing them with those of six large shallow thrust earthquakes ($M_s \geq 7.4$) of that time period, four along the coast of Oaxaca in 1928¹³⁻¹⁵ and the Jalisco earthquakes in 1932 (ref. 13). Of the seismograms available to us, those from De Bilt and La Paz (both Galitzin-Wilip seismographs) could be used for synthetic P-wave modelling¹⁶. Three point sources at depths of 40, 25 and 25 km were used to fit the observed seismograms (Fig. 3). Although it is not possible to determine in detail the geometry and the rupture process of the fault with only two stations, the experiment of synthesizing P waves shows the earthquake may be modelled as a normal fault with a focal depth of ~30-40 km. This depth is in good agreement with those of other smaller, normal-faulting events in Oaxaca and Guerrero (G.S., in preparation).

The various epicentral locations given for this event^{14,17-19} show that the 1931 earthquake occurred farther inland than the other large events. Unlike these thrust earthquakes, the 1931 Oaxaca event devastated the city and the valley of Oaxaca and caused numerous landslides¹; little damage, however, was reported along the coast and no tsunami was observed¹. The epicentre¹⁸ shown in Fig. 1 is in better agreement with the damage reports¹⁹ and is supported by S-P travel times of aftershocks recorded in Veracruz and Tacubaya (Mexico City), which show an east-west trend at about the latitude of this epicentre (F. Núñez-Cornú and L. Ponce, personal communications).

The field report of the earthquake¹ found no evidence for surface breakage. Furthermore, geologists now working in the area report no recent faulting (F. Ortega, personal communication). At a depth of 30 or 40 km, obtained from the P-wave modelling, the 1931 earthquake would lie beneath the plate contact inferred from the location of the shallow-thrust earthquake of 1978 and its aftershocks²⁰⁻²², and beneath the Mohorovicic discontinuity observed in a recent refraction experiment (C. Valdes *et al.*, in preparation) (Fig. 4). We take this evidence to suggest that the rupture during the 1931 event occurred within the subducted slab and not in the overriding plate.

The total seismic moment released estimated from the P waves is 2.3×10^{27} dyn cm and the scalar moment calculated from the Rayleigh waves recorded at De Bilt is 5×10^{27} dyn cm. An

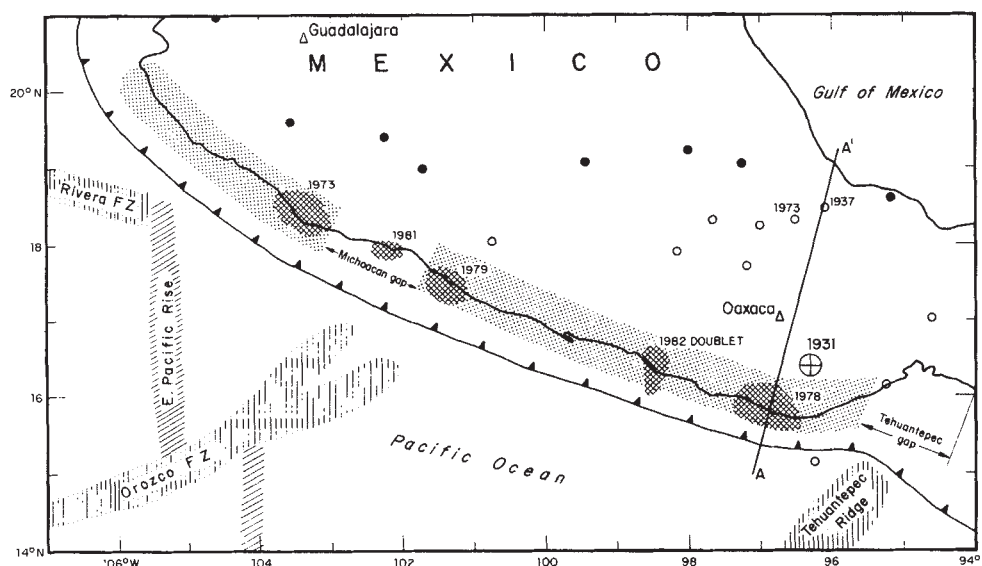


Fig. 1 Location map showing the epicentre of the 1931 earthquake (⊕). Aftershock areas of recent large shallow thrust earthquakes are shown cross-hatched. The stippled band (~80 km wide) is the maximum extent of strong coupling between the subducting Cocos plate and the overriding continent. ●, Recent volcanoes; ○, epicentres of other normal fault earthquakes with body-wave magnitude ≥ 6.0 . A section along AA' is shown in Fig. 4.

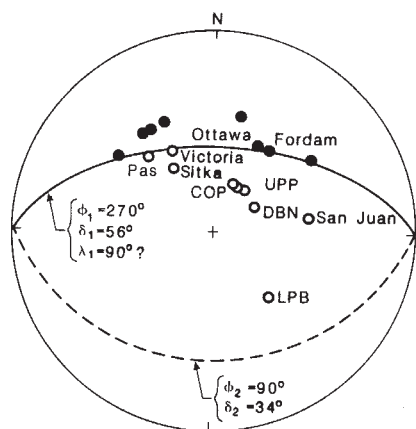


Fig. 2 First motion data of the 1931 earthquake on an equal-area lower-hemisphere projection. Dilatational (○) and compressional (●) first motions are shown. Unlabelled stations are from the Mexican Seismic Network. Other stations are: DBN, De Bilt; LPB, La Paz; UPP, Uppsala; COP, Copenhagen; STB, Strasbourg.

earthquake of this magnitude would rupture an area²³ of $\sim 7,000 \text{ km}^2$. Assuming an aspect ratio of the fault (width/length) of 0.7, the width of the fault would be $\sim 70 \text{ km}$. For a relatively young slab, such as the Cocos plate, this would mean the 1931 Oaxaca earthquake broke almost entirely through the subducted plate, decoupling it from the upper part of the slab and perhaps from the overriding continental plate.

The 1931 Oaxaca earthquake was preceded by four large thrust earthquakes ($M_s \geq 7.4$) along the coast of Oaxaca in 1928 (refs 13–15). The question arises of whether there is a causal relationship between the 1928 sequence of large thrust earthquakes along the coast of Oaxaca and the 1931 normal fault earthquake. In general, normal fault earthquakes of this size have not been observed to follow great thrust events. Nevertheless, the 1970 Peruvian earthquake, the other great normal faulting event to have occurred landward of the trench⁴, took place in spatial and temporal proximity to the thrust event of 1966. Intuitively, one would expect that the tensional stresses in the slab, near and below the plate interface broken by a recent thrust event, would be reduced by the relative plate motion on the fault. On the other hand, the gravitational pull exerted seawards of the broken plate contact by the sinking lithosphere may cause large-scale extensional stresses within the plate. Abe³ suggests this mechanism to explain the lithospheric normal

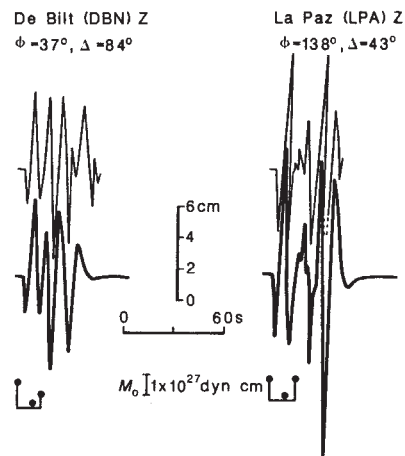


Fig. 3 Observed (upper trace) and synthetic (lower trace) seismograms at De Bilt (DBN) and La Paz (LPB). Both DBN and LPB are Galitzin-Wilip seismographs (natural period of seismometer and galvanometer $\approx 12 \text{ s}$). The largest downswing on LPB seismogram is clipped. Synthetics were generated with the focal mechanism shown in Fig. 2 and required three point sources (depths 40, 25 and 25 km). The width of the trapezoidal source time functions for each source are 2, 5 and 7 s. The seismic moments (M_0) and the time delays of the sources are indicated.

faulting event that followed the great 1965 Aleutian earthquake. In the case of the Oaxaca earthquakes, it is difficult to establish a causal relationship between the thrust and normal faulting events given that the 1931 event occurred down-dip of the recently ruptured megathrust.

The focal depths of inter-plate events in this area are consistently shallower than 20 km (refs 9, 10, 24), and the rupture areas of large earthquakes defined by aftershock studies show that in all cases the down-dip width of the seismogenic megathrust is $< 80 \text{ km}$ (Fig. 1). Even for the largest Middle-American earthquake this century, the Jalisco-Colima earthquake of 3 June 1932 (ref. 6), the down-dip fault width does not exceed that value (S.K.S. and L. Ponce, in preparation). It is possible that beyond this zone of strong seismogenic contact the subducted Cocos plate is decoupled from the overriding continental plate. Perhaps this decoupling is achieved by the breakup of the subducted slab through large, albeit infrequent, normal fault earthquakes (Fig. 1). This decoupling may explain why the Middle-American arc, a Chilean-Alaskan type of arc in

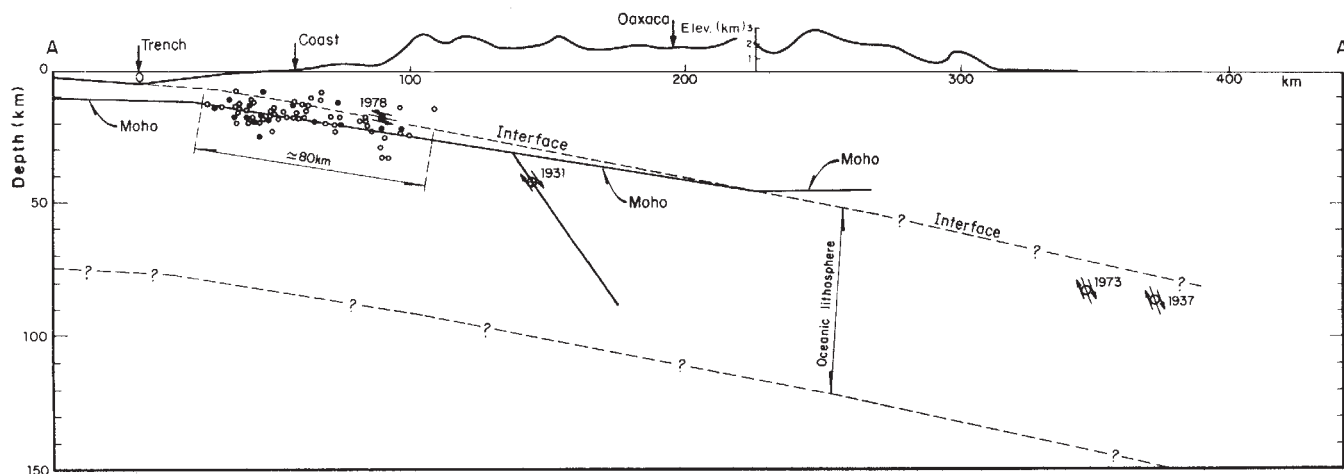


Fig. 4 A section along AA' of Fig. 1. Main shock (location and sense of slip) and aftershocks of 29 November 1978 ($M_s = 7.8$) suggest a zone of strong coupling between the plates $\sim 80 \text{ km}$ wide (refs 20–22) (stippled area in Fig. 1). Aftershocks with magnitude $M < 4$ (○) and $M \geq 4$ (●) are shown. The earthquake of 15 January 1931 ($M_s = 8.0$) was located just beyond the zone of strong coupling and probably broke the entire Cocos plate. If the normal fault earthquakes of 1937 and 1973 (ref. 29) occurred in the subducted Cocos plate, then the dip of the interface is $\sim 15^\circ$.

Kanamori's nomenclature²⁵, where young oceanic lithosphere is subducted at a fast rate, does not have a large contact zone that results in major earthquakes like those of 1960 in Chile or 1964 in Alaska. The earthquake of 19 June 1858 in northern Michoacan⁶ ($M_s \approx 7.5$) may have been another such normal fault event. It also caused heavy damage inland, but little damage was reported along the coast. Unfortunately, due to sparse population density, the epicentre of the 1858 earthquake has not been reliably determined.

It has been suggested that normal fault earthquakes within the slab reflect the gravitational pull of the down-going slab²⁶. In Mexico, however, the slab is sub-horizontal and extends no deeper than ~100 km (refs 7, 27); such a slab would probably not produce a large downward force. One mechanism that might explain the tensile stress in this young subducting oceanic lithosphere is loading by the continental plate²⁸. Whatever the mechanism, the 1931 Oaxaca earthquake shows that the geometry of the subduction zone beneath Middle America is far more complex than had been previously assumed, and that such earthquakes pose a great seismic risk because the epicentres are farther inland, where the population density is highest. Identification of earthquakes with mechanisms like the 1931 event is also important in estimating recurrence times of large thrust earthquakes and in testing earthquake recurrence models; one would not want mistakenly to include the 1931 earthquake in the analysis of shallow thrust events in Middle America.

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Segregation of efferent connections and receptive field properties in visual area V2 of the macaque

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V2 is a visual area of the macaque monkey which is at the second level in a recently proposed hierarchy of cortical visual areas¹. Histochemical staining for cytochrome oxidase (CO) in V2 reveals a pattern of alternate thick and thin CO-rich stripes separated by CO-sparse interstripes^{2,3}. These subregions receive distinct inputs from neurones in CO-rich and CO-sparse zones arrayed within the superficial layers of V1 (refs 4, 5). Are output projections from V2 to higher visual areas also segregated? Using an anatomical double-label paradigm, we have now demonstrated that V2 cells projecting to two of its major target areas, MT and V4 (refs 6, 7), are arranged in stripe-like clusters which are largely segregated from one another and which are closely related to the pattern of CO stripes. Concomitant electrophysiological recordings from V2 indicate that groups of cells having similar receptive field properties are clustered within the subregions defined by these anatomical techniques.

In four hemispheres from three monkeys, pressure injections were made into MT using the blue-fluorescing tracer bisbenzimidazole and into V4 using the yellow-fluorescing tracer Nuclear yellow. Each injection was delivered through a 1- μ l Hamilton syringe equipped with an electrically insulated barrel which was used to monitor visually evoked responses at the syringe tip¹. These recordings allowed us to identify MT in the superior temporal sulcus by its characteristic response properties⁸ and also to find the retinotopically corresponding regions in MT and in V4 on the prelunate gyrus, thereby ensuring a common region of retrograde labelling in V2. After an appropriate survival time, each brain was perfused according to the protocol of Mesulam⁹ and sectioned either in the coronal plane (three

hemispheres) or in the plane tangential to the flattened surface of V2 and V1 after post-fixation dissection¹⁰ (one hemisphere).

The pattern of retrograde labelling in coronal sections of V2 usually appeared as discrete patches of fluorescent neurones within cortical layers 2 and 3 and as a few labelled cells in layers 5 and 6 lying in register with the supragranular clusters. Figure 1 shows the distribution of the patches in one coronal section through the posterior lip of the lunette sulcus. In this particular section, V2 cells labelled from MT and V4 were segregated into alternate clusters with gaps between clusters and no regions of overlap. In most other sections from this and other hemispheres, there was some intermixing, but within the regions of overlap, one type of efferent cell was much more numerous. Convincing examples of double-labelled cells containing both Nuclear yellow and bisbenzimidazole were not observed, indicating that few, if any, cells project simultaneously to both MT and V4. Within V1, neurones labelled with bisbenzimidazole were found in layer 4B, as expected from the known V1-to-MT connections¹. CO staining in this hemisphere was only partially successful, but did permit the localization of some V4 projection clusters to thin CO stripes and adjacent interstripes.

Sections cut tangentially to the flattened surface of V2 provided a more extensive indication of the pattern of retrograde labelling and of CO histochemistry in individual sections, as well as a more accurate reconstruction of the complete pattern from several sequential sections through the region of interest. Using blood vessels and other features to align sections from such a case, the locations of all labelled cells in laminae 2 and 3 were compiled in a diagram of a single common section (Fig. 2a, b). The labelled cells cluster along rows running roughly perpendicular to the V1/V2 border at the top of the drawing. The densely populated bands of MT projection cells tend to avoid the heavy concentrations of V4 projection cells. To emphasize this, the outlines of the major MT projection bands in Fig. 2a have been added to the plot of V4 projection cells in Fig. 2b. Outside the MT projection bands, some intermixing does occur between clusters of V4 labelled neurones and scattered MT projection cells. In addition, some circumscribed regions (Fig. 2a, b, small arrows) contain few fluorescent cells

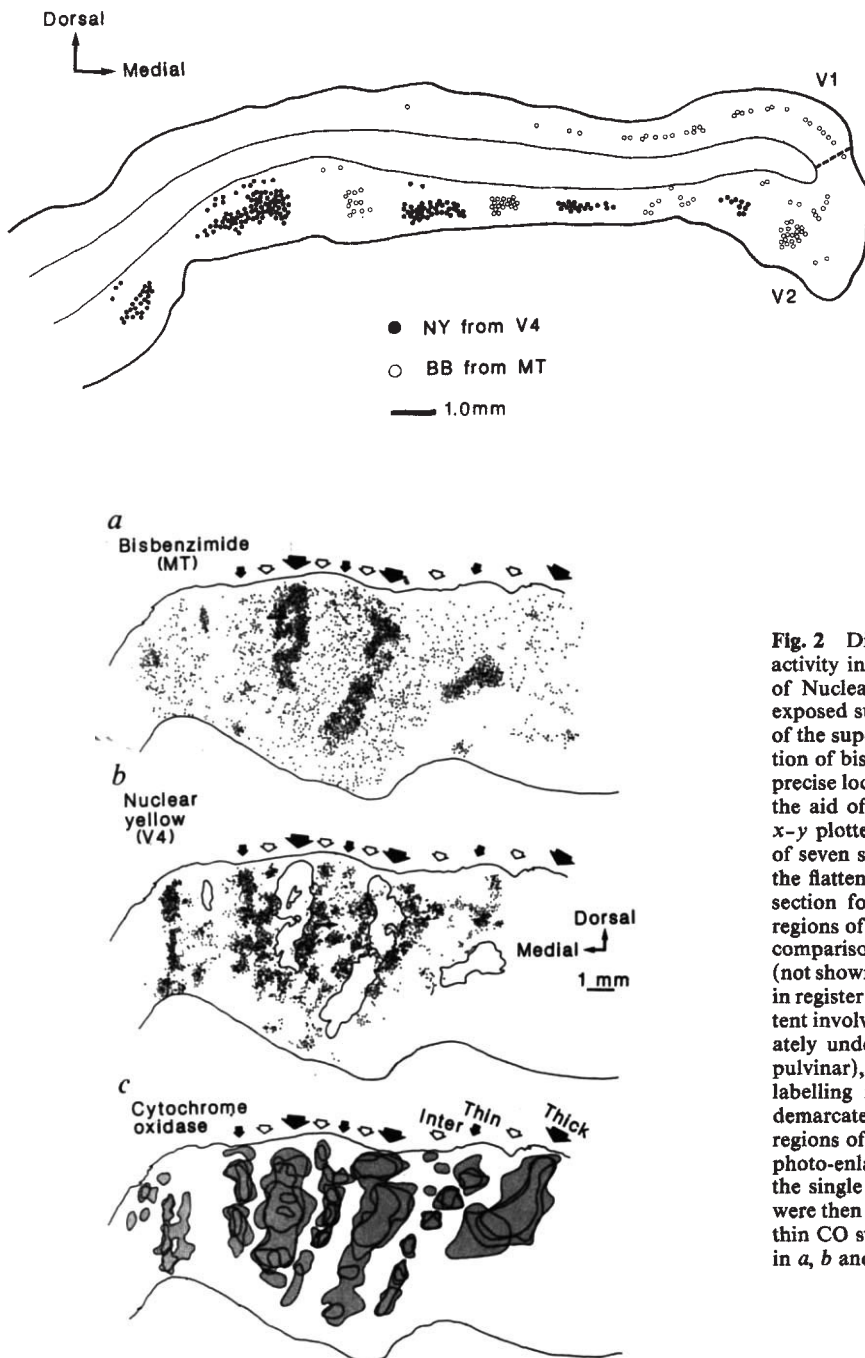


Fig. 1 Coronal section through V1 and V2, illustrating the distribution of cell bodies labeled by retrograde transport of Nuclear yellow (NY) from V4 (filled circles) or bisbenzimidazole (BB) from MT (open circles). In all cases, individual tracer injections consisted of 200 nl of a 2% aqueous suspension of Nuclear yellow (courtesy of Dr Richard Andersen) or 10% aqueous solution of bisbenzimidazole (blue fluorescing; Sigma). In histological sections, the region of heavy tracer concentration, indicated by diffuse 'haze', was 1.0–2.0 mm diameter and was confined to grey matter unless otherwise indicated. All injections were placed within a retinotopic region confined to 2°–8° eccentricity in the lower visual quadrant. The one animal with injections in both hemispheres also had a transection of the corpus callosum for other reasons. In the case illustrated here, three Nuclear yellow injections were placed at 2.0-mm intervals along the exposed surface of the anterior bank of the lunate sulcus. Area MT received one injection of bisbenzimidazole, whose location was subsequently confirmed in myelin-stained sections. Post-injection survival, 48 h.

Fig. 2 Distributions of retrogradely labelled cell bodies and CO activity in flattened V2 cortex. In this hemisphere, six injections of Nuclear yellow were placed within an 8.0-mm extent of the exposed surface of the prelunate gyrus adjacent to the confluence of the superior temporal and sylvian sulci. MT received one injection of bisbenzimidazole. Post-injection survival, 30 h. In *a* and *b*, the precise locations of individual fluorescent cells were recorded with the aid of a digitally controlled microscope stage coupled to an x-y plotter. The patterns of labelling in the supragranular layers of seven sections (30 µm thick, 120 µm apart) cut tangentially to the flattened surface were aligned and compiled onto a common section for each tracer. The outlines of the densely populated regions of bisbenzimidazole labelling have been duplicated in *b* to aid comparison. In this hemisphere the labelling in infragranular layers (not shown) was more extensive than in other cases and less closely in register with supragranular clusters. We attribute this to inadvertent involvement of a small region of injured white matter immediately under the injection site in MT (perhaps projecting to the pulvinar), and for these reasons have discounted the infragranular labelling in our interpretation of this case. Small thin arrows demarcate isolated regions having few labelled cells. In *c*, the regions of high CO activity were outlined by eye on high contrast photo-enlargements of four individual sections and compiled onto the single section shown here; overlapping areas of high density were then shaded. Large and small black arrows indicate thick and thin CO stripes; open arrows indicate interstripe regions. Arrows in *a*, *b* and *c* have been identically placed to facilitate comparison of the three patterns.

of either type, either because they project to areas other than MT and V4 or because they project to portions of MT or V4 outside the region of tracer uptake.

The relationship between the pattern of efferent cell bands and the pattern of CO staining can be seen by comparing Fig. 2*a* and *b* with *c* in which regions of high CO activity from sections passing through the infragranular layers have been compiled onto the same diagram used to display the fluorescent labelled cells. Consistent with earlier reports^{2,3,5,11}, the CO-dense regions form irregular stripes which are alternately thick and thin and are separated by interstripe regions of low activity. The densely populated bands of MT projection neurones are centred above the thick CO stripes (Fig. 2*a*, *c*, thick arrows), whereas V4 projection cells are concentrated above the adjacent interstripes and in some portions of the thin stripes (Fig. 2*b*, *c*, open and thin arrows). Several of the V4 projection clusters also encroached up to 750 µm past the border of the thick stripes as

delineated by our methods (see Fig. 2 legend). Considering all three hemispheres in which efferent clusters could be assigned to CO stripes, six densely labelled MT projection bands were associated with thick stripes, two sparsely labelled, irregular patches were associated with thin stripes, and one small, irregular patch was associated with an interstripe. For V4 projection bands, three were centred on thin stripes and five on interstripes, whereas none were associated with the central 1 mm of thick stripes.

To determine whether cells in the different CO subregions and efferent clusters share distinctive receptive field properties, we advanced microelectrodes in 150-µm steps through V2 and, at each site, recorded multi-unit or single-unit responses to bars of light projected under computer control onto a tangent screen. Quantitative estimates were made of the selectivity for direction of movement, flashed orientation, colour, and binocular versus monocular stimulation. Three important findings emerge from

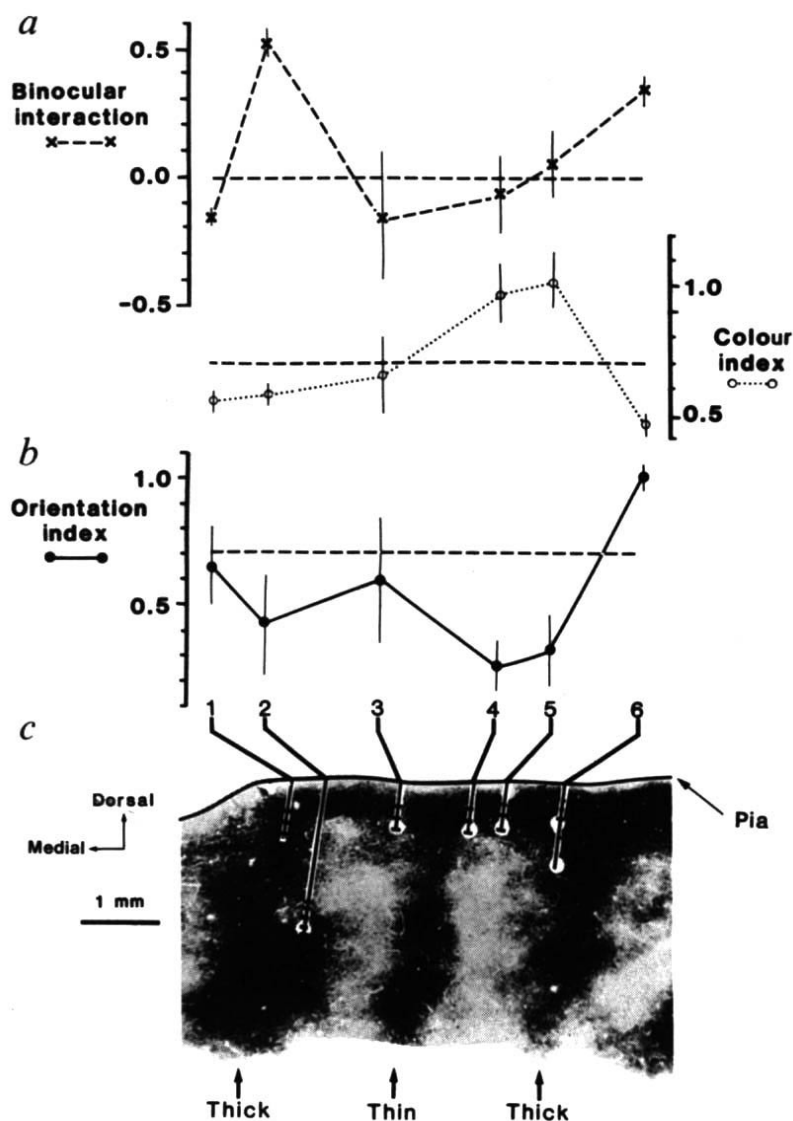


Fig. 3 Stimulus-selective neuronal activity within different CO subregions of flattened V2. At each crossbar on the electrode track diagrams in *c*, multi-unit responses (MR) were quantified by using a window discriminator to count the number of times the recorded neural signal crossed a pre-set threshold during the stimulus presentation. The rate of spontaneous background activity in the 2 s preceding the stimulus was subtracted to yield the final value. Visual stimuli were bright bars moving across or, for orientation selectivity, flashed onto a white tangent screen. Luminances: tangent screen background 10–20 cd m^{-2} , white bars 2.0 log units above background; equiluminant narrow band (interference filter) colour bars 1.8 log units above background. Selectivity indices: index of binocular interaction (assessed at the best disparity) = $1 - (\text{largest monocular MR} / \text{binocular MR})$; colour index = $1 - (\text{MR worst } \lambda / \text{MR best } \lambda)$; orientation index = $1 - (\text{orthogonal orientation MR} / \text{best orientation MR})$; direction index (not plotted here) = $1 - (\text{null direction MR} / \text{best direction MR})$. *a*, *b*, Average selectivity indices for the three to five recording sites in each penetration. Error bars signify $\pm$ s.e.m. Horizontal lines provide reference for identifying highly selective index values. *c*, CO (darker regions) stained section cut tangentially to the flattened surface of cortex, consisting of mainly subgranular layers except at the dorsal margin where, because of cortical curvature, the section passes through more superficial layers, which appear uniformly dark in this photo. Although each penetration traversed several adjacent sections, all recording sites have been compiled onto this single section and are indicated by crossbars on track diagrams. The superficial portion of penetration 2 was in V1, not evident in this section. White circles indicate marking lesions. The recording sites of penetrations 1, 2, 6 are in thick CO stripes and, based on this and adjacent sections, those of 3, 5 are in interstripes. Those of 4 are in the edge of a thin stripe. Thick/thin labels on the dark CO stripes were assigned on the basis of stripe width in this and adjacent sections and on the basis of the overall alternation of thick and thin stripes in the complete section.

the 315 recording sites studied: (1) there is significant clustering of neurones selective for each of the four parameters tested; (2) the distribution of selected responses is related to CO/efferent cell subregions, but (3) each anatomical subregion is not completely homogeneous with regard to the physiological properties represented therein.

Figure 3 illustrates results from an experiment in which six short penetrations were placed into V2 in a manner that ensured sampling from a full cycle of CO stripes. The CO-stained section illustrating the stripe pattern (Fig. 3*c*) was cut tangentially to the flattened cortical surface, except at the upper margin (see Fig. 3 legend). From this and nearby sections, recording sites in each penetration could be assigned to a specific CO subregion. Figure 3*a*, *b* displays the average selectivity indices for the three to five recording sites in each penetration for each of three stimulus parameters. For colour and orientation, index values >0.7 indicate strong selectivity; for binocular interaction, values >0.0 indicate that binocular responses were stronger than either monocular response (see Fig. 3 legend). Clusters of cells preferring binocular stimulation were found only at the recording sites of penetrations 2 and 6, both assigned to thick CO stripes; penetration 6 also showed clustering of orientation selectivity. Colour-selective sequences were observed only in penetrations 4, passing through the edge of a thin stripe, and 5, passing through an interstripe; orientation selectivity was particularly low in both penetrations. Strong direction selectivity (not shown) was present only for two recording sites in a thick stripe at the

end of penetration 2. Heterogeneity in multi-unit response properties within a CO subregion can be seen by comparing penetrations 1, 2 and 6 (all in thick stripes) and penetrations 3 and 5 (both in interstripes).

The overall incidence of selectivity for all multi-unit recording sites tested was 39% for colour, 10% for direction, 48% for binocular interaction and 41% for orientation (Table 1). (Values for a population of single units may, of course, be somewhat different.) Strong clustering was found for each type of selectivity. The majority of selective sites occurred in runs of three to nine, except for direction selectivity, for which one-third of the sites occurred in runs of two to four. Based on discrete probability estimates, clustering for all parameters was statistically significant ($P < 0.001$).

A total of 158 recording sites were accurately localized with respect to the CO stripes, fluorescent efferent clusters or both, assuming that each system extends radially through the cortex. The incidence of selectivity encountered in CO and efferent cell subregions is indicated in Table 1. For all assignable sites tested for colour, the incidence of selectivity was high in thin CO stripes, interstripes and V4 projection clusters (mean 70%), but was significantly lower in thick stripes and MT projection clusters (mean 19%). Of the few colour sites in thick stripes or MT projection patches, none occurred in clusters. Though rare overall, the incidence of direction-selective sites was markedly higher for MT than for V4 projection clusters. The differences were less striking for direction selectivity versus CO subregion.

However, all clustered, direction-selective sites occurred in thick stripes or MT projection patches. All subregions contained some sites preferring binocular stimulation, but their incidence was high only in thick stripes and MT projection patches. Strong orientation selectivity occurred in nearly half of the sites in thick CO stripes and MT projection clusters. It was also common in V4 projection clusters, although it was comparatively rare in thin stripes and interstripes. Whether this apparent discrepancy between V4 projection clusters and CO thin and interstripes reflects a sampling bias, or an interesting functional heterogeneity, remains to be determined.

The results presented here confirm that the projections from V2 to MT originate in discrete clusters¹ which are related to thick CO stripes¹², and they show that the projections to V4 arise from a separate population of cells associated mainly with thin stripes and interstripes. There may also be room to sequester additional clusters of efferent cells, such as those projecting to V3^{6,13} or back to V1. The segregation of efferent clusters with respect to the pattern of CO labelling complements the recent

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Cyclic GMP-sensitive conductance in outer segment membrane of catfish cones

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Table 1 Percentage incidence of strongly selective responses within V2 subregions

Stimulus Parameter	V2 subregion					
	Overall	Cytochrome oxidase			Efferent zone	
		Thin	Inter	Thick	MT	V4
Colour	39 (105/272)	86* (18/21)	64* (14/22)	16* (6/38)	22* (2/9)	67* (34/51)
Direction	10 (30/415)	7† (2/27)	0† (0/22)	19† (3/13)	62* (8/13)	2* (1/64)
Binocular	48 (55/114)	33* (6/18)	22* (7/32)	68* (28/41)	80† (4/5)	24‡ (6/25)
Orientation	41 (115/284)	20‡ (4/20)	17‡ (4/27)	51‡ (20/39)	40 (4/10)	42 (27/65)

Incidence of selective responses is shown as the percentage of highly selective recording sites out of all sites which (1) were assignable to the specified subregion and (2) were tested for selectivity to the specified parameter. The actual number of selective sites out of these which were localized and tested is shown in parentheses. The overall incidence values are based on all sites tested for the specified parameter, including those sites that could not be assigned to a specific subregion. A response was classified as strongly selective if the selectivity index (see Fig. 3) was >0.7 for colour, direction and orientation and >0.3 for binocular interaction. Statistical significance of χ^2 tests of the departure of obtained percentages from a uniform distribution at the 'Overall' percentage: * $P < 0.001$; † $0.1 > P > 0.05$; ‡ $P < 0.05$.

observations that V1 projections, pulvinar afferents and intrinsic V2 connections also distribute selectively to the different CO subregions^{2,4,5,14}. That these subregions are physiologically as well as anatomically distinct is indicated by the present electrophysiological results as well as the 2-deoxyglucose results of Tootell *et al.*³. Altogether, these findings suggest that there are at least two functional subregions within V2; one has V4 as a major target and may be heavily involved in the processing of colour but also is concerned with aspects of stereoscopic and orientation information; the second, projecting to MT, deals with certain (perhaps different?) aspects of stereopsis and orientation, but also processes motion information. Thus, these subregions may elaborate upon the functionally distinct inputs arriving from V1 and contribute not only to the distinctive specialization of MT for motion and V4 for colour but also impart to both the information needed to analyse other, three-dimensional properties such as location and form. Similar results have recently been reported by Shipp and Zeki¹⁵ and by Hubel and Livingstone¹⁶.

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A cyclic GMP-sensitive conductance has recently been observed with patch-clamp recording in excised inside-out patches of plasma membrane from frog and toad rod outer segments^{1,2}. This conductance has properties suggesting that it is probably the light-sensitive conductance involved in visual transduction^{1,2}. We now report a similar conductance in the outer segment membrane of catfish cones. Cyclic GMP showed positive cooperativity in opening this conductance, with a Hill coefficient of 1.6-3.0 and a half-saturating cGMP concentration of 35-70 μ M. Cyclic AMP at 1 mM, or changing Ca concentration (in the presence of Mg), had little effect on the conductance. In physiological solutions the cGMP-induced current had a reversal potential near +10 mV; the current amplitude increased roughly exponentially with membrane potential in both depolarizing and hyperpolarizing directions. Our results suggest that cGMP is also the internal transmitter for phototransduction in cones.

In visible light, a patch pipette containing normal Ringer's solution was sealed against the tip of the outer segment of an intact catfish cone, establishing a Gigaohm membrane patch³. The bath solution was then switched from Ringer's to a pseudointracellular solution (Fig. 1 legend), and a gentle tap on the micromanipulator supporting the pipette yielded an excised, inside-out membrane patch³, with its cytoplasmic face exposed to perfusion.

Figure 1A shows current recordings from a patch. In the absence of cGMP, the potential between the inside and the outside of the pipette was clamped at 0 mV, and superposed on this were voltage steps of 1 s duration at ± 10 mV increments. In the presence of 420 μ M cGMP a small inward current appeared at 0 mV; at the same time the voltage steps resulted in larger current jumps. Associated with the cGMP-induced current there was an increase in current noise, which was especially pronounced at large depolarizations and hyperpolarizations. However, no obvious opening and closing of individual channels could be resolved up to 1 kHz, indicating either an unusually small unit conductance, as has been suggested in rods^{1,4-6}, or extremely brief channel dwell-times in the conducting state. Figure 1B shows, on an expanded timescale, that the conductance turned on and off as rapidly as the change in cGMP concentration. The speed of solution change was inferred from junction currents⁷ recorded on switching from the pseudointracellular to Ringer's solutions.

Figure 2A shows the current-voltage relation (filled circles) for the cGMP-induced current of Fig. 1. The current had a reversal potential of +15 mV, and it increased steeply with voltage in both depolarizing and hyperpolarizing directions.

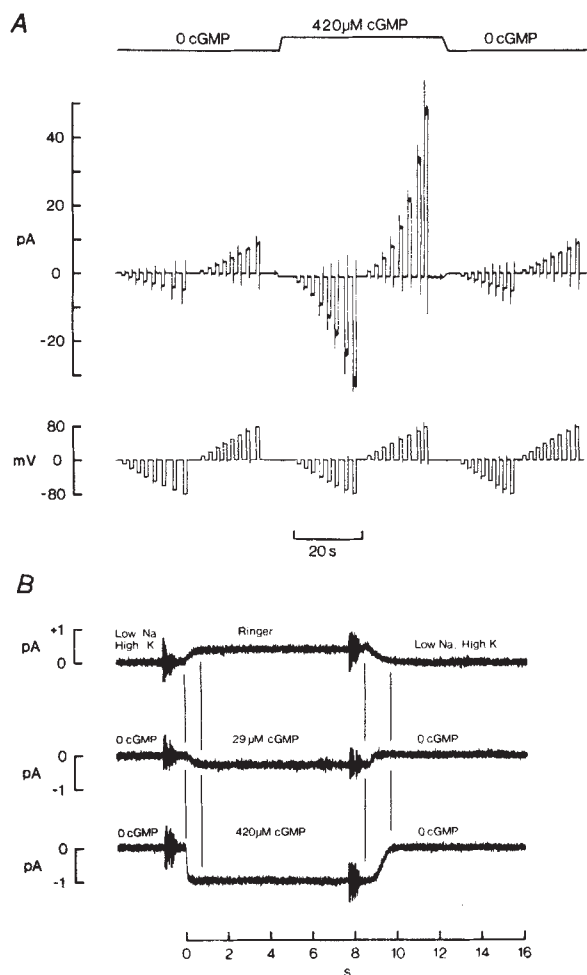


Fig. 1 Cyclic GMP-sensitive current recorded from an excised, inside-out membrane patch from a catfish cone outer segment. A piece of retina from a dark-adapted catfish (*Ictalurus punctatus*) was incubated in darkness for 1 h in Ringer's solution containing 1 mg ml^{-1} hyaluronidase (Sigma Type I-S), then finely chopped under infrared light to yield many isolated cone receptors with intact outer and inner segments. Subsequent recordings were made in visible light. There were most probably two classes of cones in this preparation²⁶; however, in the absence of any obvious morphological distinction between them the cones were selected randomly and treated as one class. The patch pipette had a tip lumen diameter of $0.5 \mu\text{m}$ or less and was connected to a Dagan 8900 patch-clamp recording system in voltage-clamp mode. Sealing against cell membrane was established by mouth suction. Room temperature. Normal Ringer's solution contained (in mM): 110 NaCl, 2.5 KCl, 1.6 MgCl_2 , 1.0 CaCl_2 , 5 TMA (tetramethylammonium)/HEPES, pH 7.6. Pseudointracellular solution contained (in mM): 12.5 NaCl, 100 K gluconate, 1.6 MgCl_2 , 0.1 TMA/EGTA, 5 TMA/HEPES, pH 7.0. All cGMP-containing solutions also had $20 \mu\text{M}$ IBMX (isobutylmethylxanthine). The IBMX was routinely added to inhibit any possible residual phosphodiesterase activity in the preparation, although control experiments without IBMX indicated that this was unnecessary. In other experiments using different concentrations of IBMX we have also verified that IBMX by itself has no agonistic effect on the cGMP-sensitive conductance, nor does it inhibit the action of cGMP. Solution changes were controlled electronically so that they could be reproduced many times. All recordings shown here were low-pass filtered at 150 Hz (8-pole Butterworth). **A**, Top, current recordings (current entering pipette from bath was positive); bottom, monitor of voltage pulses. Time course of solution changes was based on junction current measurements⁷ on switching bath solution from pseudointracellular to normal Ringer's solutions (see **B**). Each voltage pulse lasted 1 s and was switched on manually. The nature of the time-dependent change in current after a voltage jump (especially for the large depolarizing pulses) was unknown; it was, however, similar in the absence and the presence of cGMP, suggesting it was not associated with the cGMP-sensitive current. The capacity current transients occurring at some of the voltage jumps have been missed by the digitization. **B**, Time course of the turning on and off of the cGMP-dependent current closely followed the admission and removal of cGMP. Same experiment as **A**; all single sweeps; 0 mV. Upper trace, junction current measurements upon switching from pseudointracellular solution back to Ringer's. Middle and lower traces, turning on and off of cGMP-dependent currents. (All changes in current produced by cGMP shown here reflected genuine cGMP-sensitive currents because in experiments on unresponsive patches cGMP up to $840 \mu\text{M}$ gave undetectable junction currents.) The faster turning on, and delayed turning off, of the current with $420 \mu\text{M}$ cGMP were most probably due to the fact that most of the conductance was already activated at cGMP concentrations much lower than $420 \mu\text{M}$ (see Fig. 4). The noise cluster appearing before each solution change was caused by vibration from tap movement.

From 27 patches, the reversal potential was $13.5 \pm 2.9 \text{ mV}$ (mean $\pm$ s.d.). This agreed with the reversal potential for the light-sensitive current in rods^{6,8-10}, but was somewhat more positive than that estimated in tiger salamander cones (-7.8 mV ; see ref. 11). The steep increases in current at large depolarizations and hyperpolarizations were observed in all experiments. Figure 2B shows examples from four other patches. These characteristics broadly resembled those observed for the light-sensitive current in tiger salamander cones¹¹, as expected if the cGMP-sensitive conductance was the light-sensitive conductance; a quantitative comparison, however, was not possible because of the limited measurements in intact cones. The smooth curves fitted to the data in both Fig. 2A and B were scalings of the function¹²:

$$j(V) = \exp\left(\frac{(1-\gamma)(V-V_r)}{V_0}\right) - \exp\left(\frac{-\gamma(V-V_r)}{V_0}\right) \quad (1)$$

where j is transmembrane current, V is membrane potential, V_r is reversal potential, V_0 is a voltage constant and γ is a symmetry constant. One interpretation of equation (1) is that it describes current through a membrane conductance with a single energy barrier situated at a fractional distance γ (that is, γ varies between 0 and 1) from the extracellular boundary of the membrane¹². Our cone data were consistently fitted with $V_0 = 12.5 \text{ mV}$ and $\gamma = 0.35$. A voltage constant of 12.5 mV may suggest that the transmembrane current is carried by divalent cations or pairs of monovalent cations⁹. On the other hand, it may also reflect the voltage dependence of a current blockage by divalent cations (see below). In rods, unlike cones, the light sensitive and cGMP-dependent currents exhibited prominent outward rectification^{1,2,6,8-10}. However, equation (1) can still describe the rod currents, but with $\gamma \rightarrow 0$ and V_0 between 12.5 mV and 25 mV

(refs 8-10). A difference in γ may suggest that a structural group on the light-sensitive conductance which influences ion permeation is at different locations in rods and cones. A consequence of the current-voltage relation in cones is that as a cone cell hyperpolarizes in non-saturating light, the remaining light-sensitive conductance will increase and generate a larger residual dark current. This effectively reduces the cell's voltage response to light. Assuming a membrane potential in darkness of -40 mV and a ratio of light-sensitive to light-insensitive conductances of near unity¹³, it can be calculated that a hyperpolarizing response elicited at low light intensities would be $\sim 40\%$ smaller for the $I-V$ relation in Fig. 2A than for one that is flat in the hyperpolarizing range, as in rods. The difference is progressively less at higher light intensities. Such a sensitivity reduction, though sizeable, can nonetheless account for only a very small fraction of the overall sensitivity difference between rods and cones, which is about 100-fold or higher¹⁴⁻¹⁶.

Not every membrane patch that we recorded from showed a response to cGMP. Roughly 75% of the patches were responsive, and successful encounters were random; the unresponsive patches perhaps represented sealed-off vesicles³. In functioning patches that were electrically stable for 15 min or longer, the response to cGMP generally persisted upon repeated application of the chemical, with only a small progressive decline in its saturated amplitude. This observation, together with the fact that ATP was not required in the bath solution to elicit the response, suggested that cGMP acted on the conductance directly^{1,2} rather than via a protein kinase.

Figure 3A shows that a large change in Ca concentration on the cytoplasmic side of the membrane had little effect on the current in both the presence and the absence of cGMP. Figure 3B shows that the conductance was specifically activated by

Fig. 2 **A**, Current-voltage (I - V) relation of the cGMP-dependent current of Fig. 1A. Each current measurement was made at the end of the 1-s voltage pulse. Δ , ∇ , I - V relations in control solution before and after the application of cyclic GMP, with resistance of about 14 G Ω (empty electrode resistance $\sim$ 20 M Ω); $\square$, I - V relation in the presence of cGMP; $\bullet$, I - V relation for cGMP-sensitive current, obtained as difference between measurements in the presence and absence of cGMP. Smooth curve is scaling of equation (1) with $V_r = +15$ mV, $V_0 = 12.5$ mV and $\gamma = 0.35$. **B**, Normalized I - V relations for cGMP-dependent current in four other cone patches. For purpose of comparison, the data in each patch have been shifted such that the reversal potential is at 0 mV, and have been normalized such that the current at 40 mV positive to the reversal potential is unity. Actual reversal potentials and currents at 0 mV were: $\bullet$, +13 mV, -0.8 pA; $\blacktriangle$, +10 mV, -0.1 pA; $\blacktriangledown$, +15 mV, -0.5 pA; $\blacksquare$, +15 mV, -1.2 pA. Cyclic GMP concentrations were 420 μ M for $\bullet$, $\blacktriangledown$ and $\blacksquare$, and 840 μ M for $\blacktriangle$. Smooth curve is again equation (1) with $V_r = 0$, $V_0 = 12.5$ mV and $\gamma = 0.35$.

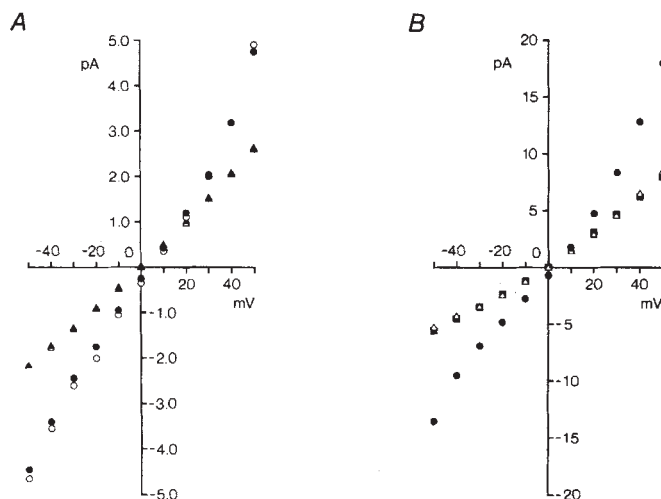
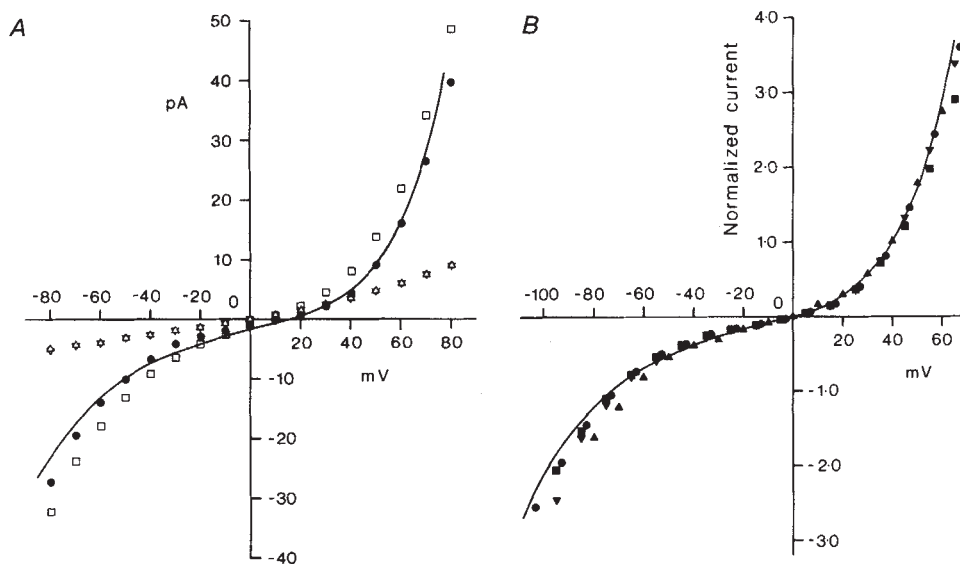


Fig. 3 **A**, I - V relations across a cone membrane patch in the presence/absence of cGMP and Ca. The bath solution contained: Δ , 0 cGMP, 0 Ca, 0.1 mM EGTA; $\blacktriangle$, 0 cGMP, 1 mM Ca; $\circ$, 420 μ M cGMP, 0 Ca, 0.1 mM EGTA; $\bullet$, 420 μ M cGMP, 1 mM Ca. Concentrations of other ions were the same as in Fig. 1 legend. **B**, I - V relations with cGMP or cAMP. Different patch from A. All solutions contained 0 Ca, 0.1 mM EGTA. Δ , Control pseudointracellular solution; $\bullet$, 420 μ M cGMP; $\blacksquare$, 920 μ M cAMP. For fair comparison, the cAMP-containing solution also had 20 μ M IBMX, though as mentioned in Fig. 1 legend the presence of IBMX was irrelevant. Data in both A and B were raw, so that the I - V relation for the cGMP-dependent current in either case would be given by the difference between the relations in the presence and absence of the chemical.

cGMP, and cAMP at about 1 mM had no observable effect. Similar observations have previously been made on the cGMP-sensitive conductance in rod membrane patches^{1,2}. It should be pointed out here that, in both rod and cone patches, Ca had little observable effect on the conductance only when Mg was present. In the absence of Mg some blockage of the conductance by Ca became apparent. It appears that Mg (and also Ca, although normally its action on the cytoplasmic side is probably masked by Mg) exerts a voltage-dependent block on the conductance, thereby influencing both the magnitude and the rectification of the macroscopic current (L.H. and K.-W.Y., manuscript in preparation). This action of divalent cations on the conductance might represent a 'flickering block' like that observed in other conductances¹⁷⁻¹⁹.

Figure 4 shows the dependence of the current on cGMP concentration at 0 mV. As in rods^{1,2}, the relation was sigmoidal, indicating that cGMP molecules had positive cooperativity in turning on the conductance. From 23 patches, the Hill coefficient ranged from 1.6 to 3.0 (mean $\pm$ s.d. = 2.4 ± 0.5), and the half-saturating cGMP concentration ($K_{1/2}$) ranged from 35 to 67 μ M

(mean $\pm$ s.d. = 55 ± 9 μ M). Both parameters were fairly similar to those previously measured from rod membrane patches^{1,2}. We found no indication of any change in the Hill coefficient with membrane potential (0 to ± 40 mV). The $K_{1/2}$ also showed very little voltage dependence, increasing by perhaps 15% from 0 to -40 mV (10 patches); this would correspond to an e -fold change over 300 mV, in a direction opposite to that expected from the negative charge on cGMP if the receptor site were located within the transmembrane electric field. The insensitivity of $K_{1/2}$ to voltage suggests that the cGMP binding site is at or very near the cytoplasmic surface of the membrane.

There has been some dispute, based on biochemical studies²⁰⁻²⁵, about whether a cGMP cascade similar to that in rods is present in cones, and there has even been a suggestion²² that cAMP in cones may assume the role of cGMP in rods. The present study, however, provides strong evidence that cGMP mediates phototransduction in both kinds of photoreceptors. Since cGMP turns on the rod and cone conductances with strikingly similar characteristics, the mechanisms underlying the large differences in light sensitivity and response kinetics

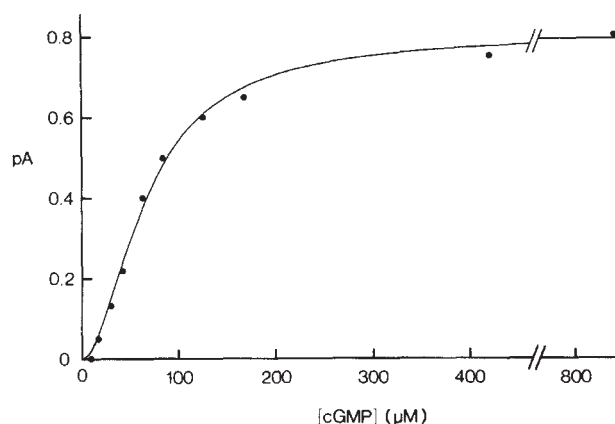


Fig. 4 Dependence of the cGMP-sensitive current on concentration of cGMP. Membrane potential clamped at 0 mV. Plotted currents were absolute values. Cyclic GMP concentrations were 8.4, 16.8, 29.4, 42, 63, 84, 126, 168, 420 and 840 μM respectively. Smooth curve fitted to the data is scaled according to the function $f(C) = C^n / [C^n + K_{1/2}^n]$, where C is concentration, $K_{1/2}$ is the half-saturating concentration and n is Hill coefficient. In this experiment $K_{1/2} = 67 \mu\text{M}$ and $n = 1.8$.

between the two kinds of receptors¹⁴⁻¹⁶ must reside elsewhere in the transduction chain, most probably in the light-controlled cGMP metabolism.

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Cyclic GMP increases photocurrent and light sensitivity of retinal cones

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Like retinal rods, cone photoreceptors contain cyclic GMP and light-activated phosphodiesterase¹⁻³. The cGMP phosphodiesterase cascade is thought to mediate phototransduction in rods. Biochemical assays of nucleotide content in cone-dominant retinas, however, have failed to demonstrate light-induced changes in cGMP¹. Changes in cyclic AMP following light exposure have been reported, leading to the suggestion that in cone phototransduction cAMP assumes a role analogous to that played by cGMP in rods⁴. Cyclic GMP introduced from tight-seal pipettes into isolated cones of the larval tiger salamander, *Ambystoma tigrinum*, rapidly increases light-modulated membrane current more than 10-fold. In cones, as in rods, cGMP also causes an approximately 10-fold increase in photocurrent duration and a 5- to 10-fold increase in light-sensitivity. Cyclic AMP has no effect on cone photocurrents under the same conditions. Because cGMP has similar effects on photocurrent magnitude and kinetics in both rods and cones, we conclude that cGMP plays corresponding roles in transduction in both vertebrate photoreceptor classes.

The effect of cGMP on cone photocurrents, which were always much briefer and less light-sensitive than those of rods, was assessed using methods developed for rods^{5,6}, as shown in Fig. 1. A suction electrode held the outer segment (inset) and collected light-sensitive membrane current (Fig. 1A). A patch pipette containing 5 mM cGMP formed a gigohm attached patch (AP) on the inner segment, shown by a decrease in the amplitude of current pulses in the patch electrode record (Fig. 1B). Just after attachment a flash (a) produced a 25-pA photocurrent in the suction electrode only (Fig. 1A). After rupture of the membrane patch and onset of whole-cell voltage-clamp recording (WC),

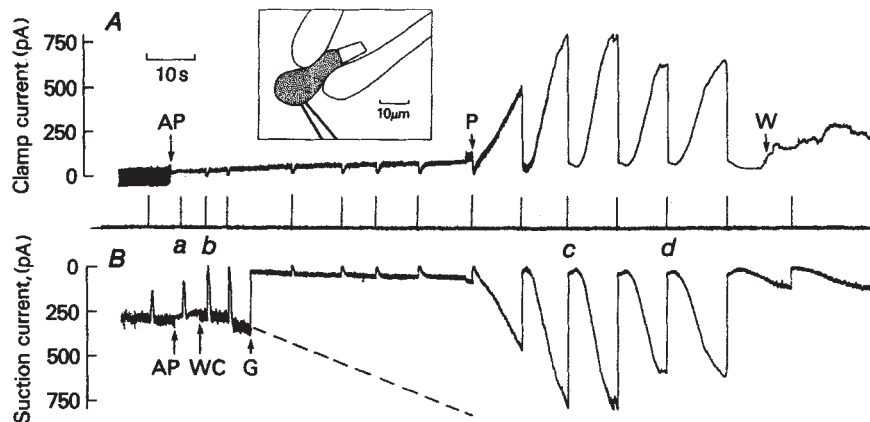
light responses appeared in both electrodes (b), marking intracellular access. Thereafter the suction pipette current and clamp current increased, first slowly and then more rapidly to ~750 pA following a brief pressure pulse in the patch pipette (P). Light flashes suppressed all current (c, d). Suction and clamp electrodes collected approximately equal photocurrents after whole-cell access, indicating little escape of photocurrent through the inner segment membrane. Photocurrent duration (time to recover 10% of pre-flash light-sensitive current) increased from 300 ms to 3,500 ms. These cGMP-induced increases in photocurrent magnitude and duration in a cone closely parallel those in rods^{5,6}.

Increases in photocurrent magnitude and duration due to cGMP infusion were measured in 10 cones. Mean current increased from 32 (range 10-65) to 250 pA (range, 76-750); mean photocurrent duration increased from 300 (range, 80-800 ms) to 2,900 ms (range 880-3,500). Control currents and light responses were re-established following removal of the patch pipette in three cells (Fig. 3). Cyclic GMP (5 mM) produced a large, persistent increase in light-sensitive current in every cell in which both electrodes collected equal photocurrents. In three cones which did not initially respond to light, whole-cell voltage clamp with cGMP-induced large photocurrents, suggesting that these cells might have been depleted of endogenous cGMP.

Cyclic AMP (5 mM) in the patch pipette produced no change in the photocurrent responses of six cones. Photocurrents and light-sensitivity during cAMP infusion could not be distinguished from recordings obtained without nucleotide in the patch pipette ($n = 5$). Intracellular access was equivalent to that achieved in experiments with cGMP, attested by clamp settling time and approximately equal photocurrents in clamp and suction electrodes over a 500-fold range of flash intensities.

Salamander cones and rods differ conspicuously in intrinsic light sensitivity, defined as the fraction of light-sensitive current suppressed per isomerization by dim flashes (linear response range). We estimated this fraction in cones to be less than 1% of the fraction (0.01-0.05) suppressed per isomerization in rods⁷. The similar increase in photocurrent duration in cones and rods,

Fig. 1 Method of introduction of cGMP into cone. Cone membrane currents recorded simultaneously with whole-cell voltage clamp (A) and suction electrode (B). Suction electrode ordinate scale applies after point marked G, at which 10-fold gain reduction in recording was made. Dashed line shows suction electrode current continued at initial gain. Tics (middle trace) mark 20- μ s xenon flashes. AP marks time of formation of gigaseal attached patch; WC marks onset of whole-cell recording, clamp command voltage at -20 mV relative to bath; P marks pulse of pressure applied to clamp electrode; W, withdrawal of clamp electrode. Clamp electrode contained 100 mM K-Acetate, 5 mM NaCl 2 mM $MgCl_2$, 10 mM HEPES, 50 μ M EGTA and 5 mM cGMP, pH = 7.5. Cell in HEPES Ringers, 1 mM $CaCl_2$ (ref. 5); $T = 21.5^\circ C$. Each flash was equivalent to $8 \times 10^5 h\nu \mu m^{-2}$, 620 nm, polarized to coincide with chromophore transition moment (equivalence obtained by comparison with 20-ms 620-nm flashes). For estimated cone absorption cross-section of $4 \mu m^2$, each flash yielded 2×10^6 isomerizations. Experiments were monitored continuously with an infrared ($\lambda > 820$ nm) video system. Inset shows tracing of videotape record of the cone of panel A. Cone inner segment is stippled; cone outer segment is clear.



however, suggested that cGMP might increase light sensitivity in both types of photoreceptors in such a way as to preserve their relative sensitivity, despite their large baseline disparity in intrinsic sensitivity. We accordingly compared the effect of cGMP on the dim flash response of both cell types.

In a rod (Fig. 2) in which light-sensitive current grew ~3-fold following whole-cell access (WC), responses to both dim (d) and bright (b) flashes became larger and prolonged. After withdrawal of the cGMP-containing pipette (W), the responses to both flashes recovered to baseline waveforms. Dim flash responses before, during and after cGMP infusion were compared on a scale of fractional current (Fig. 2C). On this scale, responses during cGMP exposure (d_2, d_3) suppressed a greater fraction of light-sensitive current (0.6) than the fraction suppressed (0.17) by pre-cGMP controls (d_1, d_4).

In a cone (Fig. 3) a dim flash (d_1) had to produce ~1,000 times more isomerizations to achieve equivalent fractional suppression (0.19). Nevertheless, on cGMP infusion the light-sensitive current of the cone increased 4.6-fold to 250 pA, and the same dim flash (d_2, d_3) then suppressed a much higher fraction of current (0.80). Following withdrawal of patch pipette (W), maximal photocurrent subsided and light responses recovered

towards those of the initial controls, with complete recovery of sensitivity (Fig. 3C). The factor by which intrinsic light sensitivity (defined above) is increased by cGMP can be estimated from our data, using the hyperbolic relationship between peak photocurrent and flash intensity⁷. This factor of sensitivity increase was 5.4 for the rod of Figs 2 and 17 for the cone of Fig. 3. The factor for a second rod was 6.4 (maximal current increase 30–500 pA) and 6.7 for a cone (maximal current increase 14–120 pA).

Comparison (Figs 2C, 3C) of dim flash responses discloses a pattern common to rods and cones. For both cell types, soon after cGMP infusion dim flash responses (normalized by maximum photocurrent) rose at about the same initial rate as pre-cGMP controls, but diverged as rate of rise of pre-cGMP control responses declines. From this comparison of dim flash responses we conclude that the increase in light sensitivity induced by cGMP is not caused by increased gain of the step in transduction fast relative to the dim flash response. Gain increase of a fast step would cause the rising phase of the response during cGMP infusion to be steeper than the control response. A mechanism consistent with the observed pattern of responses would be the retardation of the decay of one or more intermediary steps in transduction that are common to both receptor types.

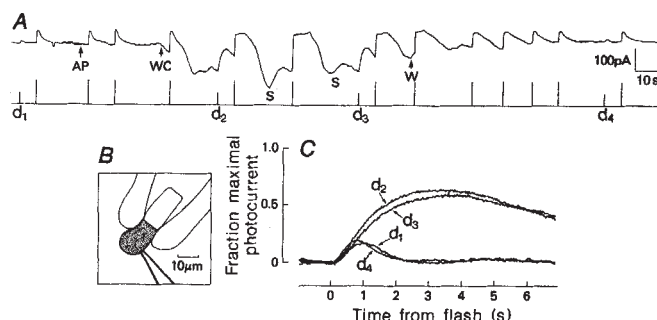


Fig. 2 Effect of cGMP on light-sensitivity of rod. A, Rod suction electrode current. AP marks attached patch; WC, whole-cell recording; S, spontaneous current fluctuations; W, withdrawal of patch pipette. Patch electrode contained 5 mM cGMP; clamp command was -40 mV. Flashes were 500 nm, 20 ms duration: dim flash (baseline, short tic mark), $0.13 h\nu \mu m^{-2}$ (~10 isomerizations); bright flash (tall tic mark), $8 h\nu \mu m^{-2}$ (~700 isomerizations). $T = 21.2^\circ C$. B, Line tracing of video record of experiment: rod inner segment stippled. C, Responses to dim flashes d_1 – d_4 from record in A. The ordinate value 1.0 represents the maximal possible photocurrent at the time of each dim flash, obtained as the difference between the current baseline immediately prior to the dim flash and the ceiling level reached by the immediately succeeding bright flash. Responses to d_2, d_3 , obtained during cGMP infusion, rise initially on common trajectory with pre- and post-cGMP control response to d_1 and d_4 .

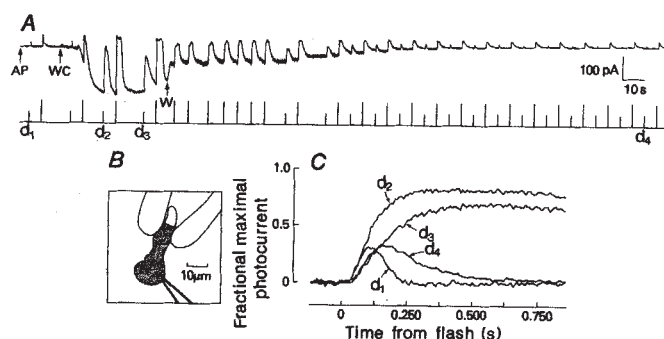


Fig. 3 Effect of cGMP on light-sensitivity of cone. A, Cone suction electrode current. AP marks attached patch; WC, whole-cell recording; W, withdrawal of patch pipette. Patching pipette contained 5 mM cGMP; clamp command was -20 mV. Flash source was xenon arc, 20 μ s duration: dim flash (baseline, short tic mark) $4,000 h\nu \mu m^{-2}$ (620 nm equivalent), yields 10^4 isomerizations for $4 \mu m^2$ collection area; bright flashes (tall tic mark), $8 \times 10^5 h\nu \mu m^{-2}$, 2×10^6 isomerizations. B, Line tracing of infra-red video record of experiment. C, Responses to dim flashes d_1 – d_4 of trace in A plotted on normalized ordinate, as in Fig. 2C. The 200 pA response to dim flash given at d_2 rises on the same trajectory as the 10 pA control response, d_1 .

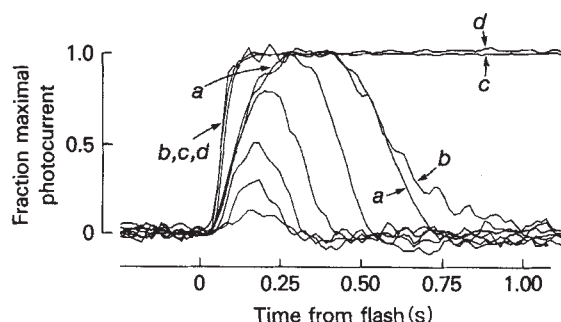


Fig. 4 Suction electrode photocurrents of cell of Fig. 1 plotted with normalized ordinate and expanded time base; ordinate value 1.0 represents maximum possible photocurrent at time of flash. Letters *a-d* identify suction electrode photocurrents of cell to flashes of 2×10^6 isomerizations; in Fig. 1 flashes are identified by the same letters. Response *b* (25 pA) obtained immediately after patch rupture, and responses *c, d* (750 pA) during cGMP infusion rise at approximately the same rate when normalized. Response *a* (18 pA), obtained before patch rupture and voltage clamping rose at slower rate. Unlabelled responses were obtained before clamping to a series of flashes graded in 0.5 log unit steps; most intense flash (*a*) equivalent to 2×10^6 isomerizations. These unclamped responses rise on a common trajectory.

Comparison (Fig. 4) of bright-flash responses of the cone of Figure 1 immediately after whole-cell voltage clamp (*b*) and later after 30-fold photocurrent increase (*c, d*) shows a pattern like that of rods⁵: large cGMP-induced photocurrent increases can occur with little change in the response rising phase.

Voltage clamp without nucleotide in the patch pipette speeds the rising phase of cone bright-flash photocurrents (data not shown). This effect can also be observed by comparing responses *a* and *b* in Fig. 4. Response *a* was obtained just before voltage clamp and response *b* immediately after clamp, but before the large cGMP-induced current. This change in the bright-flash response rising phase results from voltage clamp, and not from diffusion of solutes into or out of the cell: the slower waveform observed before voltage clamp is restored either by switching to current clamp or by withdrawal of the patch pipette. In this respect, cones differ from rods, in which voltage clamp alone has little effect on the bright-flash photocurrent. The current responsible for this behaviour of cones has not been identified.

The hypothesis that cGMP regulates light-sensitive membrane current by rapidly reversible binding to the light-sensitive conductance is supported by observations of excised patches of rod¹⁰ and cone¹¹ outer segment membranes. This hypothesis readily accounts for the large increases in light-sensitive membrane current seen on cGMP infusion in both rods and cones. Invariance of the rising phase of dim- and bright-flash photocurrents before and soon after cGMP infusion can be accounted for by adding to the hypothesis the notion that even during cGMP-infusion free cGMP in the outer segment remains much lower than the k_m of light-activated phosphodiesterase⁵. Increases of photocurrent duration and light sensitivity in both cell types, however, require a mechanism other than rapid interaction of cGMP with the light-sensitive conductance.

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Concentration of acetylcholine receptor mRNA in synaptic regions of adult muscle fibres

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Acetylcholine receptors (AChRs) are highly concentrated in the small fraction (~0.1%) of the skeletal muscle fibre surface that comprises the postsynaptic membrane of the neuromuscular junction (Fig. 1*a*). In adult murine muscle, for example, AChRs are packed at a density of over 15,000 per μm^2 in postsynaptic membrane, whereas their density is <30 per μm^2 in extrasynaptic membrane^{1,2}. Because synaptic AChRs turn over^{2,3} they must be replaced, and it is interesting to consider where the new AChRs that maintain synaptic aggregates are synthesized. One possibility is that AChRs are synthesized uniformly along the length of the multinucleated muscle fibre; in this case, AChRs might be redistributed to or selectively stabilized at the synapse, as probably occurs during synapse formation⁴⁻⁷. Alternatively, AChRs might be preferentially synthesized near synapses, a possibility that would suggest that innervation can influence not only where AChRs are inserted⁸ or accumulate but also where they are synthesized. In support of this second possibility, we report here that AChR messenger RNA is more abundant near to than far from synapses in adult muscle fibres.

Our experiment exploited the convenient anatomy of the mouse diaphragm. This thin, flat muscle is innervated by a single nerve trunk that runs perpendicular to and across the centre of the muscle fibres (Fig. 1*b*). Thus, most of the synapses (Fig. 1*c*) and the great majority of the AChRs (Fig. 1*d*) in this muscle are concentrated in its central third. We dissected diaphragms into central, synapse-rich and distal, nearly synapse-free areas, and prepared mRNA (poly(A)⁺ RNA) from each. The RNAs were fractionated on agarose gels, transferred to nitrocellulose and probed with ³²P-labelled complementary DNAs specific for the α - (ref. 9) and δ - (ref. 10) subunits of murine AChR. (Probes for the other subunits, β and γ , were not available.) The relative abundance of mRNA in the synapse-rich and synapse-free samples was measured by densitometry of the resulting autoradiographs¹¹.

Figure 2 shows that both α - and δ -subunit AChR mRNAs were severalfold more abundant in synapse-rich than in synapse-free portions of murine diaphragm. In the same experiments, we compared the abundance of actin mRNA in the two regions, using a cDNA probe specific for the skeletal muscle form of actin¹². Actin mRNA was equally distributed between synapse-rich and synapse-free samples (ratio of 1.1 ± 0.2 ; mean $\pm$ s.e.m. of six experiments), as expected for a protein present throughout the contractile apparatus of the muscle fibre. Furthermore, total yields of poly(A)⁺ RNA per g of tissue did not differ greatly between synapse-rich and synapse-free samples. Thus, the enrichment of AChR mRNA in synaptic areas is a selective phenomenon.

Interestingly, the enrichment of α -subunit mRNA in synapse-rich samples (2.9 ± 0.5 -fold; range 1.8-4.6; $n = 6$) was appreciably less than that of δ -subunit mRNA (9- and 14-fold in two experiments). This difference suggests that the transcription or processing of these two mRNA species is not regulated identically. However, the exact values we obtained may underestimate, in three respects, the actual enrichment of both AChR subunit mRNAs near synapses. First, synapses comprise only a small fraction of even the synapse-rich samples. If AChR mRNA were concentrated directly beneath the postsynaptic membrane, its level could be far higher in truly synaptic than in extrasynaptic areas. Unfortunately, we have not yet been able to detect AChR mRNA (which comprises <0.001% of poly(A)⁺ RNA in adult

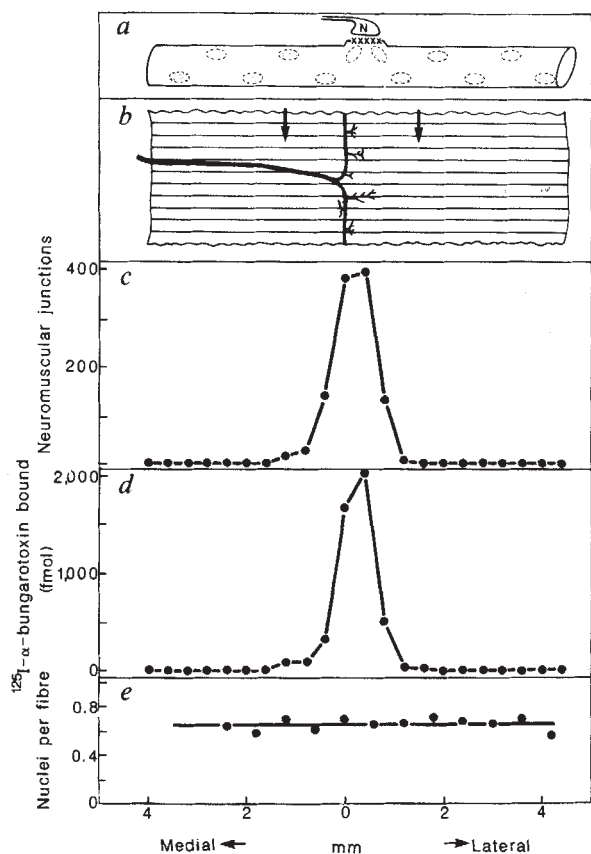


Fig. 1 Location of neuromuscular junctions and nuclei in murine diaphragm muscle. *a*, A multinucleated muscle fibre, bearing a single neuromuscular junction approximately midway along its length. AChRs (x) are concentrated in the postsynaptic membrane. Nuclei are distributed along the length of the fibre, with a few invariably beneath the postsynaptic membrane. N, nerve terminal. This sketch shows only a portion of a fibre, which, at this scale, would be ~1 m long. *b*, A strip of diaphragm muscle. Axons leave the single nerve trunk to form synapses near the midpoint of muscle fibres. Arrows indicate approximately where muscles were cut to divide them into synapse-rich and synapse-free areas. *c*, Distribution of neuromuscular junctions along a strip of diaphragm, determined by counting cholinesterase-stained endplates on serial 100- μ m sections. *d*, Distribution of AChRs in the same sections, assessed by binding of 125 I- α -bungarotoxin. *e*, Distribution of nuclei along diaphragm muscle fibres, determined electron microscopically.

Methods. *c*, *d*, A sublethal dose of 125 I- α -bungarotoxin (60 pmol; 1,000 c.p.m. fmol $^{-1}$) was injected into the thorax of a mouse³; 14 h later, the animal was killed and the diaphragm was fixed in 4% paraformaldehyde. A 5-mm-wide strip of diaphragm, intact from origin to insertion, was cut into serial, 100- μ m sections, which were counted in pairs in a γ -counter. The sections were then stained for cholinesterase¹⁴ and mounted on slides; endplates were counted at a magnification of $\times 400$. *e*, Diaphragms were fixed in 1% glutaraldehyde plus 2% paraformaldehyde, and strips were cut into measured pieces, embedded in Araldite and sectioned for electron microscopy. Myonuclei were counted in 80–120 muscle fibres per section; average numbers of nuclei per muscle fibre cross-section were plotted. Satellite cell nuclei, which were $<5\%$ as numerous as myonuclei, are not included in the counts. The figure combines data from two muscles. Using these data, a measured mean nuclear length of 11 μ m and the formula in ref. 15, we calculate that each murine diaphragm muscle fibre bears ~500 nuclei.

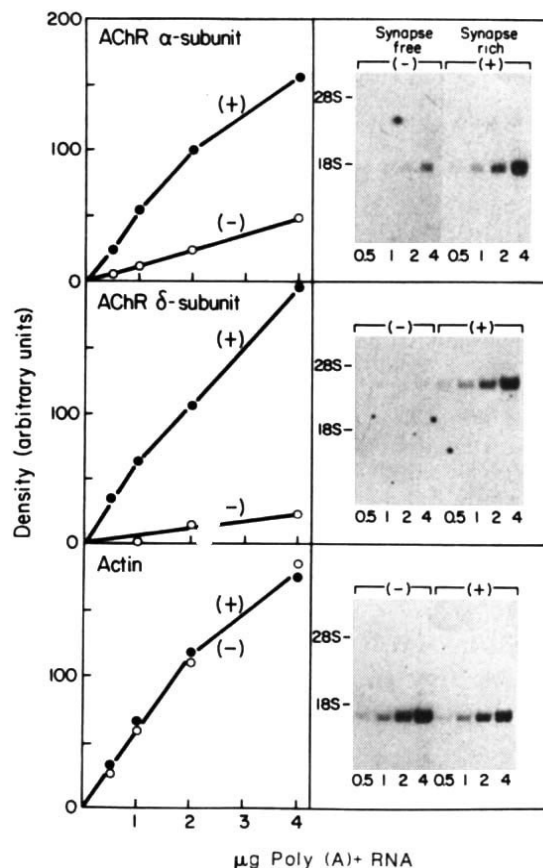


Fig. 2 Abundance of AChR α -subunit, AChR δ -subunit and actin mRNAs in synapse-rich and synapse-free segments of diaphragm muscle. Autoradiographs of Northern blots hybridized with the indicated 32 P-cDNAs are shown on the right; graphs on the left show the results obtained by densitometry of these autoradiographs.

Methods. Diaphragms from 100 adult male Swiss mice were cut into synapse-rich and synapse-free strips as shown in Fig. 1. Myotendinous junctions, which are known to bear some AChRs¹⁶ were discarded. Each muscle was dissected in ice-cold saline and frozen in liquid N₂ less than 8 min after the animal was killed; control experiments showed that levels of AChR and actin mRNA change little for at least 30 min postmortem at room temperature (not shown). Poly(A)⁺ RNA was purified from the pooled muscle, and the indicated amounts were fractionated on agarose gels, transferred to nitrocellulose and probed with 32 P-cDNA exactly as described in ref. 11. Plasmids used were pA59 for AChR α -subunit⁹, a pUC9 subclone of the 6H fragment for the AChR δ -subunit¹⁰, and p91-1 for actin¹². For A59 and 6H, cDNA inserts were excized with the appropriate restriction nuclease and purified by agarose gel electrophoresis before 32 P-labelling; 91-1 was labelled as intact plasmid. Autoradiographs were exposed for 7 days with intensifying screens for A59 and 6H, and for 12 h without an intensifying screen for p91-1. Positions of 18S and 28S ribosomal RNA markers are indicated. No nonspecific hybridization was observed with the stringency conditions used and, for each probe, species detected in synapse-rich and synapse-free samples migrated identically on gels.

difference between them. Finally, nerve branching patterns are variable, and some neuromuscular junctions might have been included in nominally synapse-free samples. Some α -subunit mRNA is clearly present extrasynaptically, in that its level in synapse-free samples is ~30% of that in synapse-rich samples. However, much of the δ -subunit mRNA detected in synapse-free regions ($\leq 10\%$ of the level in synapse-rich samples) could be attributable to imperfect dissection. Thus, AChR mRNA might be considerably more enriched in synaptic regions than our measurements indicate.

Our demonstration that AChR mRNA is concentrated near synapses in adult muscle provides strong evidence that AChRs

muscle, calculated from refs 9 and 11) by *in situ* hybridization, and therefore do not know its precise distribution. Second, we previously showed that levels of AChR α -subunit mRNA are 100-fold higher in denervated than in normally innervated adult muscles¹¹. Thus, a few denervated fibres in our muscles could have contributed significantly to the signals detected in both synapse-rich and synapse-free samples, thereby decreasing the

are preferentially synthesized in synaptic areas. The uneven distribution of mRNA could, in turn, arise in any of several ways—for example, transport of AChR mRNA from nonsynaptic to synaptic regions, increased stability of AChR mRNA near synapses or differential transcription of AChR subunit genes by nuclei near to and far from synapses. Whereas these and other alternatives remain to be distinguished, the latter is of particular interest. Although the 500 or so nuclei in each murine diaphragm muscle fibre are quite evenly distributed along the length of the fibre (Fig. 1e), a few nuclei (~5) are regularly found directly beneath the postsynaptic membrane. These synapse-associated nuclei, and the cytoplasm that surrounds them, are morphologically distinguishable from nonsynaptic nuclei and cytoplasm¹³. We speculate that AChR mRNA may be concentrated near and transcribed preferentially by these few synaptic nuclei. As only ~1% of all muscle fibre nuclei (5 per 500) are synaptic, these nuclei might transcribe orders-of-magnitude more AChR mRNA than do extrasynaptic nuclei. Thus, our results raise the possibility that synaptic and nonsynaptic nuclei within the cytoplasm of a single muscle fibre transcribe different sets of genes.

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Expression of the T-cell-specific γ gene is unnecessary in T cells recognizing class II MHC determinants

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Subtractive complementary DNA cloning combined with partial protein sequencing has allowed identification of the genes encoding the α and β subunits of T-cell receptors^{1–7}. The subtractive cDNA library prepared from the cytotoxic T lymphocyte (T_c) clone 2C has been found to contain a third type of clone encoding the γ chain^{5,8,9}. The γ gene shares several features with the α and β genes: (1) assembly from gene segments resembling immunoglobulin V, J and C (respectively variable, joining and constant region) DNA segments¹⁰; (2) rearrangement and expression in T cells and not in B cells⁸; (3) sequences reminiscent of transmembrane and intracytoplasmic regions of integral membrane proteins⁸; (4) a cysteine residue at the position expected for an interchain disulphide bond. The α and β genes are expressed at equivalent levels in both T_c cells and helper T cells (T_H)^{4,5,9,11}. The γ gene, obtained from 2C, has been found to be expressed in all T_c cells studied⁹. Here we present evidence that strongly suggests that T_H cells do not require γ gene expression.

Despite the similarities noted above, the γ gene differs from α and β in several ways: (1) clonal diversity of γ seems to be limited to the region of the V–J join (refs^{9,10}; also see below); (2) differential expression in T-cell development (γ , β and α genes are expressed predominantly early, consistently and late, respectively)¹²; (3) different chromosomal locations (mouse α , β and γ genes are located on chromosomes 14 (refs 13, 14), 6 (refs 15, 16) and 13 (ref. 13), respectively). On the basis of these similarities and differences, we postulated that the γ gene product may be a component of a T-cell receptor involved in the recognition of the major histocompatibility complex (MHC)

molecule. An interesting question is whether T_H cells, which are ordinarily restricted by the class II MHC molecules, rearrange and express the same γ gene known to be expressed in class I-restricted T_c cells. This question is particularly intriguing in light of the findings that the two types of T cell use the same J and C gene segments and probably the same pool of V gene segments in their rearranged and expressed α and β genes^{4,5,17,18}. We therefore analysed DNA from a panel of T_H hybridomas as well as the parental T lymphoma BW5147 for rearrangement of the γ gene (Fig. 1a). As was found with T_c -cell clones and T_H hybridomas studied previously^{8,9}, all demonstrated rearrangement. Northern analysis (Fig. 1b) revealed that the expression of the γ gene is variable in the T_H hybridomas, ranging from ~30% of the level of a cloned T_c -cell line, 2C, to levels undetectable even after prolonged exposure of film to the Northern filter (extended exposure not shown). No γ messenger RNA was detected in the fusion partner BW5147. By contrast, all of these cells produced comparable amounts of mRNA encoding the T-cell receptor α -chain (Fig. 1c).

To determine the reason for the irregular expression of the γ gene in T_H hybridomas, we studied four independent T_H hybridomas of different antigenic specificities which expressed γ mRNA. (T_H hybridomas B8C3, C10 and C11A3 are described in Fig. 1 legend. Hybridoma B3C6 was obtained from a CAF₁ mouse and is autoreactive (anti-I-A^d)¹⁹). From these hybridomas we prepared cDNA libraries, cloned the γ cDNA and determined the nucleotide sequences. The four T_H -derived sequences, one each from the four hybridomas, were identical and were >99% homologous at the nucleotide level to the T_c -cell (2C) derived sequence. The most significant differences between the T_H -derived and the T_c -derived sequences occur at the joining region of the V and J gene segments (Fig. 2). The 2C cDNA (pHDS 4) is encoded by the germline V108A DNA segment up to the second base of codon 99; coding by the germline J10.5 segment starts at the second base of codon 100 (ref. 10). The dinucleotide AT links the V and J segment-coded regions in the proper translation frame, and is probably provided by a germline diversity (D) segment or a terminal transferase-like enzyme. (A similar mechanism has been proposed to explain the appearance at the joining regions of immunoglobulin gene sequences of nucleotides not encoded by the germline sequence^{20,21}.) Although we have not confirmed the genomic structure of the T_H hybridoma γ genes, the limited diversity observed in the germline γ -gene components justifies our proposing the following scheme. In the T_H hybridoma, cDNA (pOE15.3) coding by the germline V108A DNA segment ends with the first base of codon 98. Germline-encoded sequence resumes in J10.5 (ref. 10) at the second nucleotide of codon 99, one nucleotide before that which begins the 2C J segment.

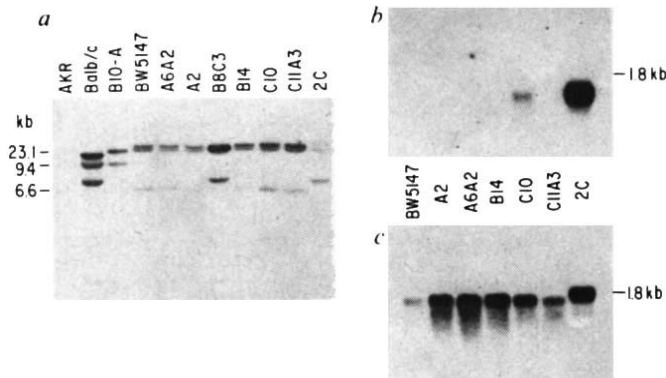


Fig. 1 *a*, Southern blot analysis²³ of T_H hybridoma DNA hybridized to a C_γ gene probe. *b*, *c*, Northern blot analyses²⁴ of poly(A)⁺ RNA from T_H hybridomas hybridized to complete γ gene (*b*) or α gene (*c*) probes. The T_H hybridoma B8C3 is of BALB/c origin and is specific for pork insulin²⁵. The T_H hybridomas A6A2, A2, B14, C10 and C11A3 are all from B10.A mice and are directed against hen egg lysozyme (ref. 26 and L.G. and Paul Allen, manuscript in preparation.)

Methods. *a*, DNA was isolated from T_H hybridomas, the fusion partner BW5147, BALB.B-derived T_C -cell clone 2C, and from livers (AKR and BALB/c) or kidneys (B10.A) of inbred mice. DNA (10 μ g) was digested with *Eco*RI and electrophoresed through 0.8% agarose gel, transferred to nitrocellulose and hybridized with ³²P-labelled nick-translated probes in 50% formamide 5 $\times$ SSC at 42 °C. Filters were washed at 65 °C in 0.2 $\times$ SSC. *b*, *c*, Approximately 2 μ g of poly(A)⁺ RNA was denatured in 50% formamide at 65 °C and electrophoresed through a gel containing 1% agarose, 6.6% formaldehyde in 20 mM sodium phosphate buffer pH 7.0. The RNA was transferred to nitrocellulose and hybridized and washed as for *a*.

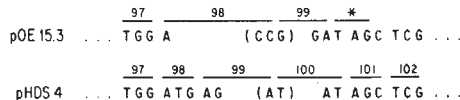


Fig. 2 Nucleotide sequences of γ cDNA clones at the V-J joint. The T_H hybridoma (B8C3) cDNA sequence is designated pOE15.3, that of the clone 2C, pHDS4. The nucleotides in parentheses are not encoded by germline sequences; their origin is discussed in the text. Numbers indicate codons, the asterisk a termination codon. All sequences were determined by the method of Maxam and Gilbert²⁷.

Between the T_H hybridoma cDNA V and J segments there are three nucleotides, CCG, which are unaccounted for by the germline sequences and which may be the product of a D segment or a terminal transferase-like enzyme. Compared with the previously determined sequences of productively rearranged γ gene (all from T_C cells), the V-J join of the T_H hybridomas alters the translational reading frame such that the open reading frame terminates immediately after codon 99. Thus, none of the four cDNA clones isolated from as many T_H hybridomas can encode a complete γ -chain.

The cDNA libraries prepared from some of the four T_H hybridoma RNA may contain a second type of γ cDNA clone, as was found to be the case with 2C (ref. 9). To test this possibility, we exploited the fact that the tetranucleotide CCGG at the V-J junction of the aberrant T_H cDNA clones is the recognition sequence of endonuclease *Msp*I. We examined a total of 28 cDNA clones (7, 2, 10 and 9 from hybridomas B8C3, B3C6, C10 and C11A3, respectively) for the presence of the *Msp*I site and found that all carry this restriction site (data not shown). As this *Msp*I cleavage site is located in the highly variable inserted sequence, these results strongly suggest that none of the four T_H hybridomas contains γ mRNA other than the one represented by the sequenced cDNA clones.

The presence of a unique, non-functional V-J joint in the γ genes of four independently isolated T_H hybridomas of different

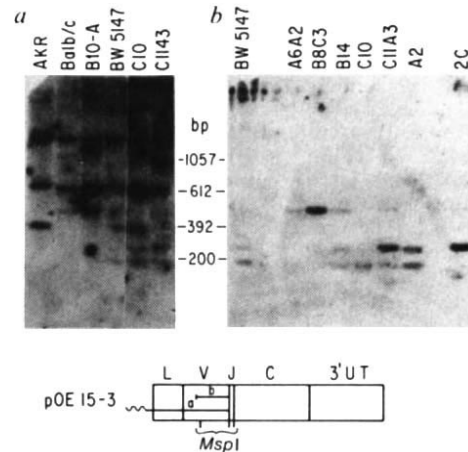


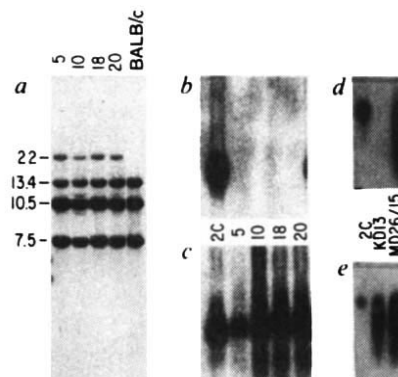
Fig. 3 Southern analyses of T_H hybridoma DNA digested with *Msp*I. The T_H hybridomas are described in Fig. 1 legend. Approximately 15 μ g of DNA was digested with *Msp*I and electrophoresed in a 1.8% agarose gel. For *a* the DNA was transferred to diazophenylthioether paper (Schleicher & Schuell) and hybridized as recommended by the manufacturer. Transfer and hybridization for *b* was to nitrocellulose as described in Fig. 1 legend. The probes used are indicated, as are the relevant restriction sites in clone pOE15.3. L and 3'UT indicate the leader region and 3' untranslated region respectively.

specificity is striking and is best explained by their having a common origin. The rearranged γ alleles of the fusion partner BW5147 are the most likely source. Although this hypothesis is in apparent conflict with the Northern blot data (Fig. 1b), it is possible that fusion with a splenic T_H cell activated the dormant BW5147-derived allele for transcription. The following result strongly suggests that this hypothesis is correct.

The functionally rearranged V_γ gene segment expressed in 2C as well as its germline counterparts contains a single *Msp*I site at nucleotide 303 (amino-acid residues 36-37). The nucleotide sequences of the T_C -cell-derived cDNA clones, as well as the germline J-C clone, indicate that the next downstream *Msp*I site on the productively rearranged γ gene is located at nucleotide 551, at the boundary of the J region and the intron following it^{9,10}. These two *Msp*I sites would yield a band of ~250 nucleotides (precisely 248 for 2C) in the *Msp*I-digested, T_C -cell clone-derived DNA hybridized to a V_γ probe. The *Msp*I site created at the aberrant V-J joint is at nucleotide position 488 of the coding sequence. *Msp*I digestion of DNA containing this rearrangement would result in a V_γ-hybridizing band 185 nucleotides in length. Figure 3 shows a Southern analysis of DNA from BW5147, T_H hybridomas and 2C. In BW5147 and the T_H hybridomas examined, the 185-nucleotide band characteristic of the aberrant rearrangement is present; as expected, no such band is present in the 2C DNA. These results indicate that the γ RNA seen in some T_H hybridomas probably arises from the aberrantly rearranged allele of the fusion partner BW5147. Although the significance of the activation of the BW5147 γ gene in some hybridomas is obscure, the absence in any of these T_H hybridomas of γ mRNA capable of encoding a complete γ protein, and the total absence of γ mRNA in some T_H hybridomas suggests that the putative γ polypeptide chain is not necessary for the function of T_H cells.

One way to confirm the proposal that γ gene expression is unnecessary in T_H cells is to analyse T_H clones (rather than T_H hybridomas), thereby avoiding the complications arising from the fusion partner. Although these T_H clones are relatively difficult to obtain, we derived several likely candidates from a mixed lymphocyte culture. These cells are negative for cell-surface antigen Lyt2 and positive for antigen L3T4, and proliferate and secrete interleukin-2 (IL-2) when stimulated by cells of the appropriate haplotype; all these are characteristics of T_H cells. Southern analysis of these cells (Fig. 4a) indicates that

Fig. 4 *a*, Southern analysis of T_H -cell clones. The cloned T_H -cell lines 5, 10, 18 and 20 were obtained from a BALB/c anti BALB.B mixed lymphocyte culture. DNA was digested with *Eco*RI and treated as described in Fig. 1*a* legend. The filter was hybridized with a C_γ probe. *b*, *c*, Northern analysis of T_H -cell clones and T_C -cell clone 2C. The methods used were as described for Fig. 1*b*. Probes used: *b*, C_γ ; *c*, C_β . *d*, *e*, Northern analysis of T_C hybridomas KD13 and MD26/15 and T_C -cell clone 2C. The KD13 hybridoma resulted from the fusion of BW5147 with a product of a C3H anti-BALB/c mixed lymphocyte culture. KD13 expresses antigens L3T4 and I-fal but not Lyt2. Following stimulation by irradiated I-E^d-bearing stimulator cells, KD13 lysed I-E^d-bearing target cells and did not produce detectable levels of IL-2. MD26/15 is described in ref. 22. Probes used were: *d*, C_γ ; *e*, C_β . **Methods.** A modification of the methods of Goldberg²⁸ and Bond and Farmer²⁹ was used. Approximately 15 μ g of total cellular RNA was electrophoresed in a gel containing 1.8% agarose, 2.2% formaldehyde in a buffer of 40 mM MOPS, pH 7.2 and 50 mM sodium acetate, pH 7.0. After electrophoresis the gel was soaked briefly in $10 \times$ SSC and transferred to nitrocellulose. Hybridization was performed as described in Fig. 1*a* legend. Probes used: *c*, C_γ ; *d*, C_β .



each clone contains an *Eco*RI fragment of 22 kilobases (kb), resulting from rearrangement of the γ genes. The other fragments were identical to those found in the embryonic configuration. Previous hybridization analyses of T_C -cell clones¹⁰ indicated that this 22-kb band, present in all T_C cells, contains C-region sequences but no detectable V-region sequence and that a 16-kb fragment, also present in all T_C cells, is representative of a productively rearranged γ gene. This 16-kb band was not found in the clones with T_H phenotype. More significantly, Northern analysis (Fig. 4*b*, *c*) of the RNA derived from these T_H clones showed normal levels of β mRNA but no indication of γ mRNA, supporting the contention that γ gene expression is not essential in mature T_H cells.

The disparity of expression observed in the two major types of T cells, T_C and T_H , of γ mRNA capable of yielding a full-length polypeptide is in striking contrast to the ubiquitous and comparable expression of the α and β gene in the two cell types. It is consistent, however, with the hypothesis that the role of the γ polypeptide chain is to recognize the class I MHC gene product. An alternative possibility is that the γ -chain is involved in effector function (that is, cytotoxicity). As a first attempt to distinguish between these two possibilities, we analysed RNA from a rare class II MHC-specific T_C hybridoma, KD13 (J.L.G. *et al.*, manuscript in preparation; see Fig. 4 legend), as well as a T_C hybridoma (MD 26/15)²² conventionally restricted to class I; neither hybridoma expresses Lyt2. As shown in Fig. 4*d*, the class II-specific hybridoma contained no detectable γ mRNA whereas the class I-restricted hybridoma did. As a control, the same RNA blot was analysed, after washing, with a β -gene probe. The autoradiogram showed a comparable level of β mRNA in both hybridomas (Fig. 4*e*). This result is consistent with a role of the γ -chain in the recognition of the class I MHC gene product rather than in the effector function, although more class II-specific T_C cells and also some class I-specific T_H cells must be analysed before a firm conclusion can be drawn.

Thus, the γ gene, productively rearranged and expressed in T_C cells and immature thymocytes, is not necessarily expressed in class II MHC-restricted T_H hybridomas, alloreactive T_H clones and in a class II MHC-specific T_C hybridoma. These results, together with the limited clonal diversity observed in this gene, suggest that the role of its gene product is in the recognition of class I MHC molecules. This recognition may occur during antigen presentation (MHC restriction) or during the intrathymic selection of self-MHC-restricted T-cell subpopulations (thymus education) or both. One implication of this hypothesis is that the class II-restricted T cells (mostly T_H cells) and/or their precursor thymocytes express a fourth type of gene,

the product of which is involved in recognition of the class II MHC gene products. If this is so, this gene has diverged too much to be detected with the probes available.

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Note added in proof: We have recently found a new V_γ gene segment which does not cross-hybridize with the previously described V_γ gene segments. This new V_γ gene segment is rearranged to a J_γ gene segment in the 22-kb *Eco*RI fragment.

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The *ras* oncogene product p21 is not a regulatory component of adenylate cyclase

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Harvey (Ha-MSV) and Kirsten (Ki-MSV) murine sarcoma viruses induce tumours in animals and transform various cells in culture because of the expression of the *ras* oncogene product, p21 (ref. 1). Proto-oncogenes homologous with these genes are highly conserved evolutionarily² and activated *ras* oncogenes have been detected in many human cancers³⁻⁷. Whether *c-ras* oncogenes are directly responsible for human carcinogenesis is uncertain; however, it is clear that p21 mediates virus-induced transformation, although by an unknown mechanism. Epithelial and fibroblast cell lines transformed with Ha-MSV and Ki-MSV express p21 (ref. 8) and exhibit reduced adenylate cyclase activity^{9,10}. Like the guanine nucleotide regulatory proteins, N_s and N_i , which mediate stimulation¹¹ and inhibition¹², respectively, of adenylate cyclase, p21 is a membrane-associated GTP binding protein, which exhibits GTPase activity¹³⁻¹⁵. These similarities suggest that p21 and the adenylate cyclase regulatory proteins are related in cellular function, and that p21 depresses adenylate cyclase by inhibiting the activity of N_s or acting as N_i . We have therefore now examined the structural and functional similarities between p21 and N_s and N_i and find no evidence that p21 regulates adenylate cyclase activity by acting as one of these regulatory proteins.

Figure 1 shows that an anti-p21 monoclonal antibody (Y13-259) immunoprecipitated detectable amounts of ³²P-ADP-ribosylated N_s (lane c) and N_i (lane g) as compared with the controls with normal IgG (lanes a, e). The recovery, however, was quite low, as the intensity of the bands was far less than the input controls (1/20th of total inputs were applied to lanes d and h). When immunoprecipitates were washed with 0.1%

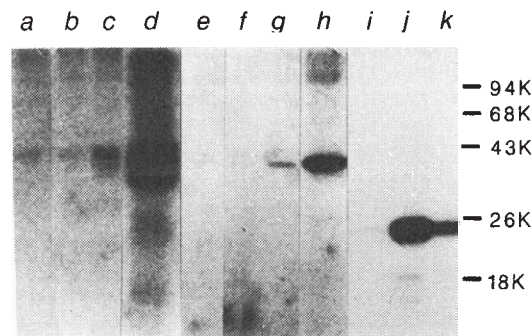


Fig. 1 Immunoprecipitation of ADP-ribosylated N_s and N_i . N_s and N_i of Ha-MSV-transformed MDCK membranes were ADP-ribosylated with cholera or pertussis toxin, respectively, using ³²P-NAD (NEN). Membrane proteins were extracted with 50 mM Tris-HCl pH 7.5, and 1% Triton X-100. Cholera toxin ADP-ribosylated protein (N_s) (75 μ g) was immunoprecipitated at 4 °C with 1.2 μ g of either normal rat IgG (lane a) or anti-p21 monoclonal antibody Y13-259 (lanes b, c). Purified p21¹⁶ (13 μ g) was added to reaction b. Immunoprecipitates were washed four times at 4 °C with Tris-buffered saline containing 1% Triton and analysed by 12% polyacrylamide gel electrophoresis in the presence of 0.1% SDS¹³. One-twentieth of the cholera toxin-labelled protein was directly applied to the gel (lane d). The same series of experiments was carried out with pertussis toxin ADP-ribosylated protein (N_i) (lanes e-h). Purified p21 (65 ng) was autophosphorylated with ³²P-GTP (270 Ci mmol⁻¹) and immunoprecipitated in the presence (lane i) or absence (lane j) of purified p21 (13 μ g). ³²P-pp21 (3 mg) was directly applied to the gel (lane k). The gel was fixed, dried and subjected to autoradiography for 30 days (lanes a-h) or 3 days (j-k) at -70 °C. M, markers are shown on the right.

SDS and 1% sodium deoxycholate, the bands were almost undetectable, indicating weak interaction.

A purified p21 overproduced in *Escherichia coli* retains full biochemical properties of GTP/GDP binding, autokinase and GTPase¹⁶. We found that this purified p21 blocked the weak immunoprecipitation of N_s (Fig. 1, lane b) and N_i (lane f), indicating that the cross-reaction was specific to p21; in similar conditions, ³²P-labelled *E. coli* p21 was almost completely precipitated (lane j). The fact that p21 has some sequence homology to GTP binding proteins, which are related to the adenylate cyclase regulatory proteins^{17,18}, may explain the very weak cross-reactivity. Similar results were obtained with other anti-p21 monoclonal antibodies, YA6-172 and Y13-238 (ref. 19). However, polyclonal antisera to p21 from tumour-bearing rats¹, and three mice monoclonal antibodies against *E. coli* p21 (S. Showalter and T.Y.S., unpublished observation), did not significantly immunoprecipitate N_s or N_i .

Despite the weak cross-reactivity of N_s and N_i with anti-p21 antibodies, p21 did not serve as a substrate for cholera or pertussis toxin-catalysed ADP-ribosylation. Although functional p21 was present in Ha-MSV-transformed MDCK cell membrane preparations, there was no protein of the electrophoretic mobility of p21 [relative molecular mass (M_r) 21,000 (21K)] which was labelled by ³²P-NAD in the total membrane lysates (Fig. 1, lanes d, h) or in the anti-p21 immunoprecipitates (lanes c, g).

To determine if p21 itself has N_s function, we performed membrane reconstitution experiments using S49 *cyc*⁻ membranes (Table 1). This mutant of S49 lymphoma cells lacks N_s protein and thus serves as a convenient system to measure functional N_s activity²⁰. The concentration of p21 in Ha-MSV-transformed MDCK cells is ~0.3–0.5 pmol per 50 μ g membrane protein. In the reconstitution experiments of Table 1, 250 pmol of purified *E. coli*-derived p21 was used in each reconstitution with 50 μ g S49 *cyc*⁻ membranes. Despite this 500-fold excess of p21, there was no detectable adenylate cyclase activity (Table 1), in conditions where MDCK membrane N_s preparations were effective in restoring activity to *cyc*⁻ membranes. Furthermore, excess p21 had no effect on the ability of N_s preparations from parental or Ha-MSV-transformed MDCK membranes to

Table 1 Reconstitution of p21 with S49 *cyc*⁻ membranes

Donor	pmol cAMP per min per mg <i>cyc</i> ⁻ membranes
None	0
p21	0
Parental MDCK(X)	5.25 ± 0.55
+p21	5.66 ± 0.44
Ha-MSV MDCK(X)	5.97 ± 0.40
+p21	5.69 ± 0.36
<i>c-ras</i>	0
<i>v-ras</i>	0

Membranes (75 μ g) from parental and Ha-MSV-transformed MDCK cells were solubilized with 1% sodium cholate and their catalytic activity was inactivated (X) by incubation at 25 °C 30 min before reconstitution with S49 *cyc*⁻ membranes as described elsewhere²⁰. *E. coli*-derived p21 (p21) and p21 purified¹ from parental (*c-ras*) and Ha-MSV-transformed membranes (*v-ras*) was treated identically. The amount of p21 per reconstitution was 250 pmol p21, 0.3 pmol *v-ras* or 0.8 pmol *c-ras*. All samples contained identical concentrations of detergent and buffer in the final reaction. Adenylate cyclase activity of the reconstituted hybrids was measured in the presence of 80 mM HEPES pH 8.0, 0.1% bovine serum albumin (BSA), 10 mM MgCl₂, 1 mM dithiothreitol (DTT), 0.5 mM ATP, 1 mM theophylline, 50 μ M GTP, 1 mM EDTA, 1 mM cyclic AMP, 2.6 mg ml⁻¹ phosphocreatine, 0.4 mg ml⁻¹ creatine phosphokinase and 10 μ M isoprenaline. Data represent pmol cAMP per min per mg *cyc*⁻ membranes of triplicate determinations ± s.d. There was no detectable activity of inactivated (X) MDCK membranes.

Table 2 Adenylate cyclase activity of membranes following pertussis toxin treatment

	Basal	PGE	NaF
	(pmol cAMP per min per mg)		
Parental MDCK	7.1 ± 0.4	15.3 ± 0.3	14.6 ± 1.4
+PT	7.3 ± 0.1	18.7 ± 0.5	17.8 ± 0.5
Ha-MSV MDCK	2.5 ± 0.2	3.8 ± 0.3	8.9 ± 1.0
+PT	2.4 ± 0.2	3.7 ± 0.4	9.0 ± 0.2
Parental NRK	14.8 ± 0.7	25.4 ± 0.8	40.7 ± 4.0
+PT	21.9 ± 0.7	38.1 ± 1.4	61.0 ± 1.2
Ki-MSV NRK	13.1 ± 0.4	14.7 ± 0.3	20.2 ± 1.2
+PT	17.0 ± 0.4	18.9 ± 0.3	24.9 ± 1.3

Membranes were treated with or without 10 µg ml⁻¹ activated (1 mM DTT, 20 min, 30 °C) pertussis toxin (PT) in 150 mM NaPO₄ pH 7.4, 10 mM thymidine, 0.5 mM ATP, 50 µM GTP, 1 mM NAD for 15 min at 30 °C. The reaction was terminated with cold 25 mM HEPES pH 7.5, and membranes were centrifuged and resuspended in 20 mM Tris pH 7.5. Adenylate cyclase activity was measured as described elsewhere⁹. Data represent the average of triplicate determinations ± s.d.

Table 3 Effect of p21 on MDCK adenylate cyclase activity

	Adenylate cyclase activity	
	Basal	100 µM forskolin
	(pmol cAMP per min mg protein)	
Parental MDCK	0.49 ± 0.02	7.7 ± 0.6
+p21	0.45 ± 0.03	10.9 ± 0.8
Ha-MSV MDCK	0.33 ± 0.03	3.2 ± 0.2
+p21	0.33 ± 0.02	4.2 ± 0.3

E. coli-derived p21 (250 pmol) was reconstituted into (50 µg) parental or Ha-MSV-transformed MDCK cell membranes as in Table 1. Adenylate cyclase activity of the reconstituted hybrids was measured in the absence (basal) or presence of 100 µM forskolin. Data represent the average ± s.d. of triplicate determinations.

reconstitute isoprenaline responsiveness of 249 cyc⁻ membranes (Table 1), excluding an indirect effect of p21 on the function of N_s.

We performed parallel reconstitution experiments with p21 purified from parental (*c-ras*) and Ha-MSV-transformed (*v-ras*) cell membranes (Table 1). It is known that p21 is post-translationally processed in mammalian cells by fatty acid modification, presumably to facilitate membrane association²¹. Although p21 overproduced in *E. coli* has full biochemical activities, it is not processed by fatty acid modification. However, as seen with *E. coli*-derived p21, neither *c-ras* (0.8 pmol per 50 µg membrane) nor *v-ras* (0.3 pmol per 50 µg membrane) p21 reconstituted adenylate cyclase activity of S49 cyc⁻ membranes.

A more likely explanation for the decreased adenylate cyclase activity of Ha-MSV-transformed MDCK cell membranes is an increased expression of N_i. However, N_i activity of transformed membranes was not significantly greater than that of parental membranes. ADP-ribosylation of N_i by pertussis toxin attenuates its activity²², resulting in increased adenylate cyclase activity. When MDCK cell membranes were ADP-ribosylated with pertussis toxin (Table 2), there was a small (20%) increase in prostaglandin E- and NaF-stimulated activity in parental but not transformed membranes. In parallel experiments with NRK cells, toxin treatment increased basal, PGE- and NaF-stimulated activities by 50% in parental and 30% in transformed membranes.

Similarly, when a 500-fold excess of purified p21 was reconstituted into parental and Ha-MSV-transformed MDCK cell membranes (Table 3), there was no change in either basal or forskolin-stimulated adenylate cyclase activity in either membrane. A decrease in adenylate cyclase activity of parental membranes in the presence of p21 would be expected if p21 functioned as N_i. Coupled with the finding that pertussis toxin had little effect on transformed cells, the data suggest that p21 does not decrease adenylate cyclase activity by acting as N_i.

The similarities between p21 and other GTP binding proteins, including the adenylate cyclase regulatory proteins, have suggested that these proteins are functionally related. Such speculation has been supported by studies with yeast²³ which demonstrated that both human and yeast *ras* genes restored adenylate cyclase activity to *ras*⁻ yeast mutants. Although p21 shares some sequence homology^{17,24,25} and slight immune cross-reactivity (Fig. 1) with N_s and N_i, the present studies find no evidence to indicate that p21 regulates mammalian adenylate cyclase. This conclusion is supported by studies with purified adenylate cyclase components. Purified p21 was not significantly ADP-ribosylated by cholera toxin (R. Kahn and A. G. Gilman, personal communication), in conditions optimal for labelling N_s (ref. 26). Additionally, a 10,000-fold excess of purified p21 had no effect on adenylate cyclase activity (R. Kahn and A. G. Gilman, personal communication) in a functional assay²⁷ of purified α/β interactions. Adenylate cyclase regulation in mammalian cells seems to differ from that of yeast. For example, there is no known ligand that regulates adenylate cyclase activity in yeast by a receptor-mediated mechanism.

However, it is tempting to speculate that p21 functions in a very similar manner to that of guanine nucleotide regulatory proteins in membrane signal transduction. Evidence suggests that an entire family of GTP binding proteins, with properties similar to those of p21, regulates various signal transducing mechanisms²⁴. The GTPase activity of activated *ras* oncogenes is very much less than that of the proto-oncogenes¹⁴. If the hydrolysis of GTP to GDP is the mechanism that turns off p21 function, reduced GTPase activity in the activated p21 would result in sustained stimulation of its cellular effectors. Although the relationship between GTP binding and hydrolysis and transformation by p21 is unclear, our present studies suggest that the biochemical similarities between p21 and the guanine nucleotide regulatory proteins of adenylate cyclase are not directly responsible for the decreased adenylate cyclase activity which accompanies viral transformation. Given the important role of protein phosphorylation in regulating cell growth and function²⁸, it is not surprising that adenylate cyclase is affected by viral transformation. Our present studies suggest that as yet undetermined functions of p21 are more relevant to this consequence of transformation.

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A novel brain ATPase with properties expected for the fast axonal transport motor

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Identification of the ATPase involved in fast axonal transport of membranous organelles has proven difficult. Myosin and dynein, other ATPases known to be involved in cell motility, have properties that are inconsistent with the established properties of fast axonal transport^{1,2}, an essential component of which is readily solubilized in physiological buffer conditions rather than being stably associated with either membranous organelles³ or cytoskeletal elements⁴. Adenylyl imidodiphosphate (AMP-PNP), a nonhydrolysable analogue of ATP, is a potent inhibitor of fast axonal transport that results in a stable interaction of membranous organelles with microtubules^{1,2}. Here we report the identification and partial characterization of an ATPase activity from brain whose binding to microtubules is stabilized by AMP-PNP. This ATPase activity seems to be associated with a polypeptide of relative molecular mass (M_r) 130,000 that is highly enriched in microtubule pellets after incubation with AMP-PNP and a soluble fraction from chick brain. This novel ATPase fraction has the predicted characteristics of the motor involved in fast axonal transport. Common features between the ATPase and fast axonal transport include interaction with the cytoskeleton in the presence of AMP-PNP, ready extractability, no Ca^{2+} dependence and inhibition by EDTA^{1,5}.

Samples of microtubules⁶, incubated with a soluble fraction of chick brain, in ATP or AMP-PNP were analysed by gel electrophoresis (Figs 1, 2), by electron microscopy (Fig. 3) and for ATPase activity (see Table 1). Final pellets were resuspended in MH buffer and assayed for ATPase activity as described in Table 1. A heat-labile ATPase was found to be preferentially associated with the AMP-PNP pellet (see Table 1). Activity of this ATPase is high in the presence of 1 mM EGTA and Mg^{2+} , but inhibited if 5 mM EDTA was added instead of EGTA. The differential effects of EGTA and EDTA indicate dependence on a divalent cation, but not Ca^{2+} .

Two major differences in the composition of labelled polypeptides associated with the AMP-PNP and ATP pellets are revealed by SDS gel electrophoresis (Fig. 1a). First, the concentration of an M_r 43,000 (43K) polypeptide is significantly increased in the AMP-PNP pellet (arrow in Fig. 1). This protein co-migrates with actin in two-dimensional gels (arrow in Fig. 2) and no other polypeptide of comparable M_r increases in concentration. When samples of S2 are incubated without addition of microtubule protein and processed in parallel, the amount of actin in the pellet is the same for both AMP-PNP and ATP (Fig. 1b). Thus, the increase in the amount of actin in the pellet is dependent on the presence both of microtubules and of AMP-PNP.

The second major difference is a highly enriched 130K polypeptide in the AMP-PNP pellet (arrowheads in Figs 1, 2). The 130K polypeptide is a novel microtubule associated protein: it is not readily detectable in cycled microtubules from rat or baboon brain or in taxol-polymerized microtubules from chick brain (Fig. 1b). It appears in the pellet only if both microtubules and AMP-PNP are present (Fig. 1). Under the conditions of the present experiments, the 130K protein is in a soluble form and becomes stably associated with microtubules only in the presence of AMP-PNP. A similar protein has been detected in pellets prepared from rat brain by the same procedure (data not shown).

A few additional minor differences in polypeptide composition can be detected, but these differences are more variable. Significantly, no differences were detected in the high M_r regions of the gel ($>200\text{K}$), where myosin and dynein, if present, would appear. Both ATP and AMP-PNP promote release of myosin

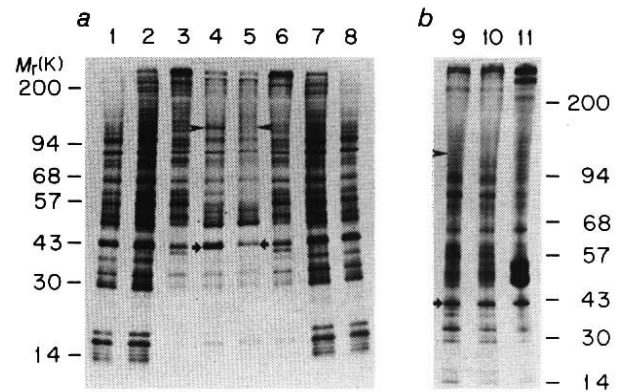


Fig. 1 Electrophoresis analysis of labelled polypeptides in AMP-PNP and ATP microtubule fractions. **a**, Four fractions were taken from each sample tube: top half of the sample (lanes 1, 8), bottom half of the sample including the interface between sample and sucrose (lanes 2, 7), sucrose fraction (lanes 3, 6) and pellets (lanes 4, 5). Lanes 1–4 are from a sample incubated with 10 mM AMP-PNP; lanes 5–8 were incubated with ATP. Matching pairs (1/8, 2/7, 3/6 and 4/5) were loaded for equal counts. Little or no qualitative difference can be detected between the pairs of supernatant fractions (1/8, 2/7 and 3/6). However, two major differences are apparent between the AMP-PNP pellet (lane 4) and the ATP pellet (lane 5): an increase in the relative amount of a 43K protein in the AMP-PNP pellet (arrows) and the appearance of a 130K band with AMP-PNP, but not ATP (arrowheads). The absence of a dramatic difference in the 130K range for the soluble fractions of the ATP emphasizes the amount of enrichment for this polypeptide in the AMP-PNP pellet. **b**, Lanes 9 and 10 show the pattern of labelled proteins in the pellets when no unlabelled microtubule protein is added to the sample. No differences are detected between AMP-PNP (lane 9) and ATP (lane 10) pellets. Lane 11 is a sample of the labelled microtubule protein from chick brain polymerized by taxol. Note that although labelled proteins corresponding in mass to the high M_r and τ microtubule-associated proteins are visible, no band corresponding to the 130K ATPase is detected. A few additional minor differences can be noted between lanes 4 and 5 (for example, a band of $\sim 60\text{K}$ is enriched in the AMP-PNP pellet), but these are less consistent features of the pellet fractions.

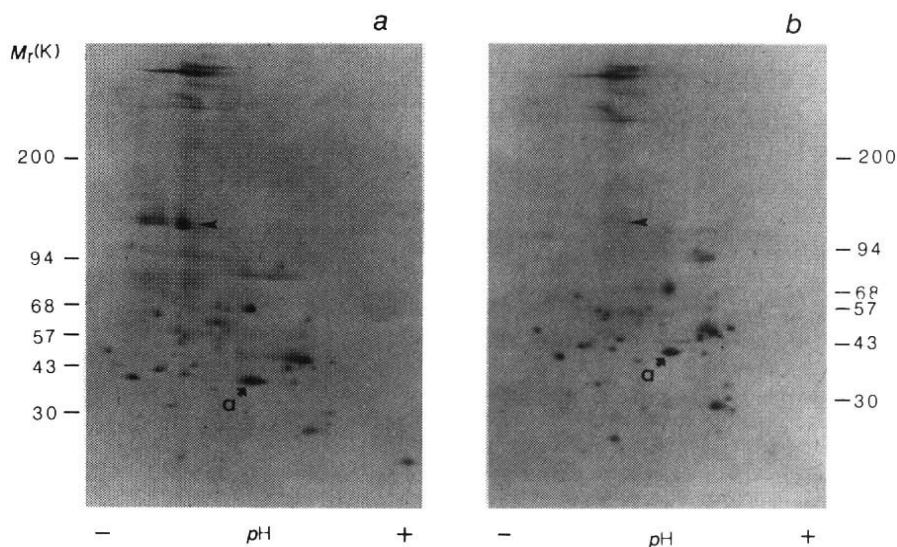
Methods. The brains of 2–7-day-old chicks were injected with 1 mCi of ^{35}S methionine in 25 μl physiological saline and 4–6 h after injection, the chicks were killed and the brains homogenized in two volumes of cold MH buffer (0.1 M PIPES, pH 6.94; 1 mM EGTA, 1 mM MgCl_2 , 1 mM GTP and 0.5 mM ATP). The homogenate was incubated on ice for 30 min, then centrifuged for 60 min at 32,000 g at 4°C. The supernatant was made 10 μM in taxol, incubated at 37°C for 30 min and centrifuged at 40,000 g for 30 min at 25°C. All endogenous labelled tubulin and microtubule-associated proteins are effectively removed from the supernatant (S2) by this step. The microtubule-depleted supernatant (S2) was combined with an equal volume of unlabelled baboon or rat brain microtubule protein (prepared as described in ref. 6) in MP buffer containing 20 mM AMP-PNP or 20 mM ATP to give a final concentration of 0.1 M PIPES (pH 6.94), 1.0 mM GTP, 0.25 mM ATP, 0.5 mM MgCl_2 , 10 μM Taxol and either 10 mM AMP-PNP or 10 mM ATP. After incubation for 30 min at 37°C, aliquots were prepared for centrifugation by layering onto a cushion of 1.0 M sucrose in MP buffer containing either 10 mM AMP-PNP or 10 mM ATP as appropriate. Samples were centrifuged for 180 min at 114,000 g at 25°C. Soluble proteins were precipitated with methanol and all pellets were resuspended in 0.5% SDS, 8 M urea and 2% 2-mercaptoethanol for analysis on a 6–17.5% polyacrylamide slab gel by a modification of the method of Laemmli¹⁸. Labelled polypeptides were visualized by fluorography¹⁹. Some samples were also analysed on 4–17.5% slab gels which give better resolution of high M_r polypeptides.

from actin⁷ and dynein from the microtubule B subfibre^{8,9} at the concentrations used in these experiments, so no significant differences in myosin or dynein content would be expected between AMP-PNP and ATP pellets.

In some experiments, the pellets were fixed and processed for electron microscopy as described in Fig. 3 legend. The AMP-PNP microtubules (Fig. 3a, c) are 'decorated' with many, occasionally regularly spaced sidearms. In addition, a considerable amount of electron-dense matrix material and more frequent membranous profiles are present. These are distinct from the less frequent sidearms and overall clean appearance of the ATP microtubules (Fig. 3b, d; ref. 10).

The correlation between increased ATPase activity and enrichment of the 130K protein suggests that this polypeptide represents a component of the ATPase protein. Actin is the only other protein that is comparably increased, but it does not have a suitable ATPase activity. Therefore, it is probable that the

Fig. 2 Two-dimensional gel electrophoresis of labelled polypeptides in *a*, AMP-PNP and *b*, ATP pellets. Samples prepared as described in Fig. 1 for SDS gel electrophoresis were diluted in 2 vol of lysis buffer (8% Triton X-100, 1.6 pH 5-7 Pharmalytes, 0.4% pH 3-10 Pharmalytes, 5% 2-mercaptoethanol and 9 M urea). Samples were analysed using a modification of the O'Farrell method²⁰ with 6-17.5% slab gels in the second dimension. Both the major differences noted in SDS electrophoresis result from changes in a single polypeptide of the corresponding M_r . The increased amount of label at 43K co-migrates with actin (arrow). The 130K band in the ATP pellet runs as a single spot with a streak towards the basic end of the gel (arrowhead in *a*). No label is detectable in the comparable region of the gel for the ATP pellet (arrowhead in *b*).



ATPase activity associated with microtubules in the presence of AMP-PNP corresponds to the 130K protein. A partial characterization of this ATPase indicates that it does not correspond to previously described microtubule-associated ATPases. The observations are consistent with the hypothesis that the ATPase activity and the 130K protein interact with the microtubule in an ATP-dependent manner, that is, that binding of ATP (or AMP-PNP) facilitates interaction of the protein with a microtubule and hydrolysis of ATP promotes release. The result would be an ATP-dependent cycle of binding to and release of the

protein from the microtubule. With the concentration of ATP used in these studies, the ATPase activity would result in the ATPase being primarily in the released or soluble state.

The increased association of actin with the AMP-PNP pellet is particularly interesting given that agents that disrupt actin microfilaments also inhibit fast axonal transport¹¹⁻¹³; these observations are difficult to reconcile with reports that only microtubules are involved in translocation of membranous organelles^{14,15}. The precise role and form of the associated actin remains to be determined, but the evidence suggests that it plays a role in axonal transport *in vivo*. Identification of microfilaments in the pellets and the precise relationship of this actin to the 130K protein and the microtubule is an important next step.

All the observed properties of the ATPase activity are consistent with the predicted properties for an ATPase involved in fast axonal transport, such as lack of a Ca^{2+} dependence and inhibition by EDTA in concentrations sufficient to chelate Mg^{2+} in the buffer¹. In the presence of ATP, the 130K protein and associated ATPase activity are easily solubilized and show minimal stable binding to microtubules. Extraction of axoplasm in a physiological buffer that approximates the small M_r composition of axoplasm but no added ATP¹ causes a gradual loss of activity with little decoration of microtubules by vesicles^{1,2,5} and a soluble fraction of squid axoplasm has recently been shown to promote movement of vesicles on microtubules⁴. However, in the presence of AMP-PNP, the 130K protein and associated ATPase activity are stably associated with the microtubule and the vesicles are stably associated with the microtubules in axoplasm^{1,2}. Finally, note that the AMP-PNP stabilization of binding occurs in the presence of 0.2 mM ATP and 1 mM GTP, conditions that would cause dissociation of myosin from actin⁷ and dynein from the B microtubule^{8,9}. Inhibition of fast axonal transport by AMP-PNP also occurs in the presence of almost stoichiometric concentrations of ATP².

The 130K protein and associated ATPase activity are strong candidates for components of the fast axonal transport 'motor'. Experiments designed to measure the binding of this protein to the vesicle component and demonstration of movement are currently in progress. The demonstration that fast axonal transport and related processes are based on a unique mechanism, distinct from previously defined molecular mechanisms of cell motility, is a major advance^{1,2}. The experiments reported here are first steps toward identifying the molecular components of that motile machinery and understanding the molecular mechanisms of axonal transport.

(During the preparation of this manuscript, I learned that, based on our earlier work with AMP-PNP^{1,2}, Vale *et al.* have

Table 1 ATPase activity in AMP-PNP and ATP pellets

	μM phosphate released per mg of protein per min
MH buffer (EGTA, Mg^{2+})	
AMP-PNP	0.280
ATP	0.078
EDTA MH buffer (no divalent cations)	
AMP-PNP	0.056
ATP	0.032
Heat-treated sample	
AMP-PNP	0.097
ATP	0.078

The final pellets (see Fig. 1 legend) were resuspended in MH buffer containing $10 \mu\text{M}$ taxol to give a final protein concentration of $20\text{--}30 \mu\text{g ml}^{-1}$. Aliquots of sample were added to appropriate buffers containing the substrate to start the incubation. For the EDTA experiments, MH buffer was modified by elimination of MgCl_2 and replacement of the EGTA by 5 mM EDTA. In the heat-denaturation experiments, sample aliquots were heated for 15 min in a Dribath temperature block at 100°C before addition of substrate. Sample assays were stopped immediately with isobutanol to determine initial phosphate levels or incubated for 10 min at 37°C . ATPase activity was measured by determining the amount of inorganic phosphate released using the method of Marsh¹⁶. The assay was calibrated against a sodium phosphate standard. Initial ATP concentration was 10 mM in the appropriate MH buffer for all experiments. The relatively high level of ATP was used to minimize effects from residual AMP-PNP in the AMP-PNP pellet. Previous studies² showed that 10 mM ATP reversed the inhibition of movement by 1 mM AMP-PNP. As a result, all of these ATPase activities represent relative activities and are likely to be underestimates of the activity associated with the AMP-PNP pellet. Protein concentration was determined by the fluorescamine method¹⁷ for each aliquot after assay for ATPase activity. Pellets were each assayed 5-10 times and the values in the table are the means of these experiments (variance 10-20%).

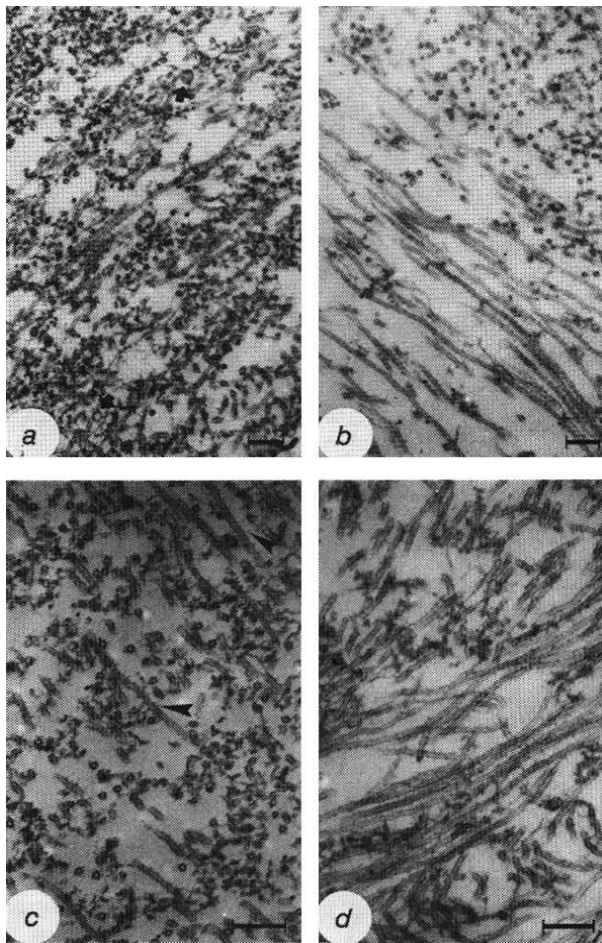


Fig. 3 Electron micrographs of microtubules and associated materials in AMP-PNP and ATP pellets. *a, c*, AMP-PNP-treated pellet; *b, d*, ATP treated pellet. The amount of material associated with the AMP-PNP microtubules (*a, c*) is much greater than with the ATP microtubules (*b, d*). The associated material includes more frequent membranous structures (arrows in *a*) and a large amount of relatively amorphous material similar to the granulofilamentous matrix associated with microtubule domains in the axon²¹. The sidearms on the microtubules also appear to differ between the two pellets. On the AMP-PNP microtubules, the sidearms are more numerous and regular in appearance (see arrowheads in *c*). The sidearms on the ATP microtubules are variable in length and less frequently observed.

Methods. All pellets were fixed for 2 h at room temperature in 2% glutaraldehyde with 1% tannic acid in 0.1 M phosphate buffer (pH 7.4). Samples were post-fixed for 1 h at room temperature in 1% osmium tetroxide in the same buffer with no tannic acid. The fixed pellets were stained *en bloc* overnight with 0.5% uranyl acetate, dehydrated through graded alcohols and embedded in Epon 812. After thin sectioning, samples were stained with uranyl acetate and lead citrate. Bars, 0.2 μ m. All micrographs were taken using a Jeol 100C transmission electron microscope.

used AMP-PNP to enrich for a similar sized protein from a soluble fraction from squid optic lobe⁴ that promotes motility in their assay²².)

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Heterogeneity of membrane phospholipid mobility in endothelial cells depends on cell substrate

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Cellular growth control and differentiation have been shown to be dependent on both cell–cell and cell–substrate contacts¹. Interactions of cells with extracellular material are critical events during embryonic development and maintenance of tissue function². Plasma membrane receptors have been described for components of the extracellular matrix such as fibronectin, laminin and various collagen types³. Transmembrane signalling has been shown to be influenced by the lateral mobilities of the plasma membrane constituents⁴. The interaction of cells with their extracellular matrix could thus have a significant effect on the mobility properties of the plasma membrane components⁵. Here we have studied the dynamic properties of fluorescent membrane phospholipids in bovine endothelial cells using fluorescence recovery after photobleaching measurements. At this molecular level we find that the phospholipid lateral diffusion coefficient is dependent on the substrate upon which cells are allowed to adhere (collagen, fibronectin or a natural basement membrane) and on the topography of the cell (basal versus apical plasma membrane).

Bovine pulmonary artery endothelial cells (BAEC) were established from primary cultures and grown in Dulbecco's minimal essential medium (Gibco) containing 10% fetal calf serum (Hyclone). We used cells between passages 3 and 10; their endothelial origin was confirmed by the presence of cytoplasmic factor VIII antigen and membrane angiotensin converting enzyme. These cells secrete a dense extracellular matrix (ECM) and exhibit polarized functional properties⁶. Cells were grown on glass coverslips pretreated by incubation at 37 °C for 30 min with a 10 μ g ml⁻¹ solution in phosphate-buffered saline of either fibronectin (Sigma) or collagen type I (Collagen Corp. or Worthington). Alternatively, cells were grown to confluence on glass coverslips, basement membranes were prepared by treatment with mild detergent and alkali⁷. Cells for experiments were allowed to adhere to these ECMs. All cells were used within 2 days after plating. A fluorescent phospholipid markers, 1 palmitoyl-2-[6-[(7-nitro-2-1,3-benzoadiazol-4-yl)amino]caproyl]phosphatidylcholine (NBD-PPC) was incorporated in the cells as described elsewhere⁸.

Experiments were performed at 10 °C in order to slow endocytosis and ensure an essentially membranal localization of the dye within the timeframe of measurements. Lateral diffusion was assessed by the technique of fluorescence recovery

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after photobleaching (FRAP)⁹ using a Ronchi ruling pattern of 133 lines per inch. In this technique an image consisting of alternating bright and dark lines is projected on a sample containing fluorescent probe molecules, to obtain a periodic variation of fluorescence intensity as a function of position. After brief laser-generated photobleaching, the subsequent redistribution of fluorescence intensity is measured with a photomultiplier and yields information about the lateral motion (for example, diffusion) of the probe molecules⁹. Diffusion coefficients (D) were calculated from the FRAP curves by fitting to a single exponential as a function of time (Table 1). For cells grown on fibronectin or collagen type I, the calculated D of NBD-PPC was very similar ($\sim 7 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$). When cells were layered on ECM, D was significantly lower ($0.96 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$). It should be pointed out that in the case of cells on ECM, the recovery curves appeared to follow a double-exponential function. In this case the D calculated by single exponential fitting represents an average coefficient.

After establishing an effect of the substrate type on the lateral diffusion properties of the lipid fluorescent probe, we wished to assess whether this effect was uniform over the cell plasma membrane. To distinguish between the fluorescent lipid mobility in the apical and basal plasma membrane, cells were grown on silicon wafers (lot no. 6263, orientation type N(100)CZ, purchased from Aurel Co. with a natural oxide layer of thickness $\leq 30 \text{ \AA}$). The basal plasma membrane is close enough to allow energy transfer from the membrane dye to the semiconductor¹⁰; when this fluorescence is quenched, only the apical membrane fluorescence contributes to the FRAP measurements.

Fluorescence quenching was investigated in detail by using a physically well-defined system. Lipid monolayers containing 2% of fluorescent dye were coated on silicon wafers with thermally grown oxide layers of varying thickness. Figure 1 shows the decrease in fluorescence intensity as a function of the silicon oxide layer thickness. For dye-silicon distances $< 200 \text{ \AA}$, the measured fluorescence intensity was reduced by more than an order of magnitude. Two physical effects contribute to the fluorescence decrease for small distances. First, the excitation intensity is reduced for distances smaller than $\lambda/4$ due to destructive interference of the incoming wave with the partially reflected wave from the silicon/silicon oxide boundary¹¹. Second, the semiconductor functions as fluorescence acceptor by energy transfer (R. Silbey, personal communication). The latter mechanism is only partially understood and is the subject of current research.

To assess both effects we overlayed the expected fluorescence excitation curve with the calculated energy transfer following a

$(R_0/d)^3$ dependence according to the planar geometry Förster equation¹⁰, where d is the distance between fluorescence donor and acceptor, in our case the silicon surface (Fig. 1). As Förster radius we assumed $R_0 = 200 \text{ \AA}$, a value that we expected to be realistic for our experimental set-up. As can be seen in Fig. 1, the resulting theoretical curve agrees well with the experimental data, supporting our estimate of the efficiency of fluorescence energy transfer from the dye to the silicon surface.

The thickness of the natural ECM is $\sim 70 \text{ \AA}$ (ref. 2). The artificial supports used in our experiments were measured by ellipsometry (data not shown) and found to have a thickness of $35 \pm 10 \text{ \AA}$ and $50 \pm 10 \text{ \AA}$ for fibronectin and collagen respectively. With a plasma membrane thickness of $80 \text{ \AA}$ and a natural silicon oxide layer of $\sim 30 \text{ \AA}$, the fluorescent lipid in the basal plasma membrane of cells grown on silicon is about $150 \text{ \AA}$ distant from the semiconductor surface and is thus quenched by more than 90%. As a control for the cells grown on the silicon wafers with natural oxide, FRAP measurements were also performed on cells grown on silicon with thermally grown oxide layers $\sim 9,000 \text{ \AA}$ thick, where no energy transfer occurs between the basal plasma membrane dye and the semiconductor. In addition, for these distances the wave interference effects are averaged by the aperture angle of illumination.

No gross morphological changes (cell shape, actin and tubulin immunofluorescence) were observed when cells were grown on the silicon surfaces. There was no significant change in the lateral mobility of the phospholipid probe for endothelial cells grown on collagen or fibronectin, both on silicon and silicon oxide surfaces compared with glass coverslips (Table 1). When cells were grown on ECM, however, there was a clear induction of asymmetry of membrane properties. Diffusion coefficients for NBD-PPC of about one order of magnitude larger ($8 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$) were measured on silicon where only the apical surface was assessed, compared with that measured on silicon oxide and glass coverslips (0.90 and $0.96 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ respectively), where both apical and basal membranes were assessed.

In the case of cells grown on ECM on silicon surfaces with a thick oxide layer, FRAP recovery curves followed a double-exponential function, in contrast to clear single-exponential FRAP curves for cells grown on all supports on silicon surfaces with a natural oxide layer. The double-exponential recovery curve doubtless reflects the heterogeneity of D . The resolution of the components of the multifunctional FRAP curves will be presented elsewhere (M.N. *et al.*, in preparation).

Note that although $\sim 35\%$ of the phospholipids were essentially immobile in the time range of our FRAP measurements, this does not imply their direct involvement in the framework

Table 1 Lateral mobility of NBD-PPC in endothelial cells grown on different supports on glass coverslips, silicon or silicon oxide surfaces

Support		Diffusion coefficient of NBD-PPC ($D \pm \text{s.d.}, \text{ cm}^2 \text{ s}^{-1}$)	Mobile fraction (% $\pm \text{s.d.}$)	No. of experiments (No. of measurements for each experiment)
ECM	Glass coverslip	$0.96 \pm 0.28 \times 10^{-9}$	68 ± 5	12 (6)
	Silicon	$8.00 \pm 0.24 \times 10^{-9}$	70 ± 4	12 (6)
	Silicon oxide	$0.90 \pm 0.27 \times 10^{-9}$	60 ± 5	12 (6)
Collagen type I	Glass coverslip	$7.00 \pm 0.21 \times 10^{-9}$	56 ± 5	12 (6)
	Silicon	$8.20 \pm 0.25 \times 10^{-9}$	60 ± 4	12 (6)
	Silicon oxide	$8.00 \pm 0.24 \times 10^{-9}$	64 ± 5	12 (6)
Fibronectin	Glass coverslip	$7.50 \pm 0.22 \times 10^{-9}$	48 ± 4	12 (5)
	Silicon	$7.50 \pm 0.22 \times 10^{-9}$	64 ± 5	12 (5)
	Silicon oxide	$7.20 \pm 0.21 \times 10^{-9}$	60 ± 5	12 (5)

Cells prelabelled with NBD-PPC on glass coverslips or silicon surfaces were mounted under phosphate-buffered saline on microscope slides with a spacer. Cells were epi-illuminated with 488-nm line of an argon ion laser (Spectra Physics 265) on a Zeiss Photomicroscope III. NBD fluorescence was detected by a photomultiplier (RCA 34103A) at -30°C interfaced to an Archives microcomputer. Photobleaching parameters were: objective 40×0.65 numerical aperture, Ronchi ruling periodicity in the objective plane $8.5 \mu\text{m}$, observation and bleaching laser powers 0.25 and 100 mW . No change in recovery time was observed with a doubling in bleach duration and no significant recovery occurred when the Ronchi ruling was removed. The diffusion coefficient (D , $\text{cm}^2 \text{ sec}^{-1}$) and the mobile fraction (%) were calculated from the slope and the amplitude of the FRAP curves.

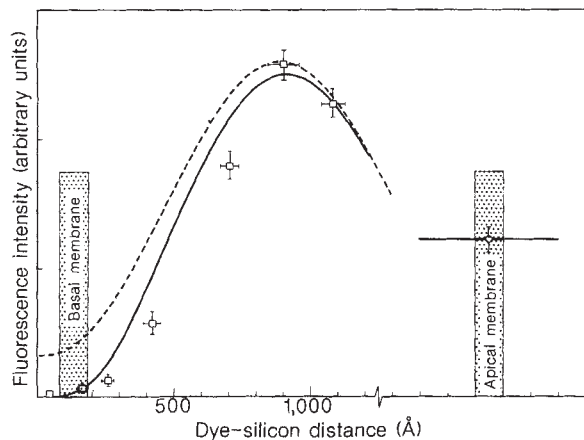


Fig. 1 Fluorescence intensity as a function of silicon-dye distances. □, The measured fluorescence intensities of an egg-PC monolayer containing 2% NBD-PPC coated on silicon wafers with various thicknesses (d) of the oxide layer. The excitation intensity (---) was calculated assuming that the phospholipid monolayer has the same refractive index as the oxide layer ($n = 1.45$). The system can thus be treated as a semiconductor ($\hat{n} = 4.36/0.07$) with a dielectric layer on the surface. The excitation of the dye is given by the superposition of the wave that is transmitted into the dielectric medium and the multiple reflections at the boundaries. Note that the dye is within the dielectric layer at the boundary to the halfspace. The intensity (I) in this case is given by¹¹: $I \approx 1 - 0.77 \cos \beta$, where $\beta = (4\pi nd/\lambda)$. Within the aperture angle of 10° , the incidence angle stays close enough to 90° so that polarization effects can be neglected. The dotted curve was multiplied with $[1 - (R_0^3/(R_0^3 + d^3))]$ to take into account the fact that the excitation is quenched by dipole interaction with the semiconductor and yields the solid curve. Fluorescence intensity was also measured on a substance consisting of a coverslip on a silicon wafer with water between them as a model for the apical membrane (○). Silicon wafers (Monsanto Co., Orientation N100) with either natural (30 Å) or thermally grown oxide layers were coated using the Langmuir-Blodgett technique at a constant pressure of 38 mN m^{-1} . Fluorescence was measured with a Spex Fluorolog in front surface mode under an aperture angle of $< 10^\circ$ with unpolarized excitation (λ_{ex} 488 nm, λ_{fl} 525 nm). Thickness was determined ellipsometrically (Gaertner L116A).

of cell-cell or cell-substrate contact. Membrane components which do not directly or even indirectly (via protein contact) interact with the substrate may still be trapped in the contact domain and thereby appear immobile.

In several cellular systems, protein mobility has been reported to be affected either by ligand-induced redistribution^{12,13} or focal contact formation¹⁴. In the latter study¹⁴, the lateral mobility of phospholipids was only changed twofold, which can be explained by the fact that only small domains of the membrane are affected. In the present study we have observed a major asymmetry in the phospholipid mobility for cells grown on ECM, presumably because a large portion of the membrane area is affected by this substrate. This asymmetry was further highlighted by the discrimination of apical membrane fluorescence.

In conclusion, we have found that both the type of polymeric support and the topography for a given cell determine the lateral mobility of the phospholipid probe. The asymmetry of the membrane properties may reflect a bifunctionality of the bovine endothelial cells used in this study. The combined use of different ECM components, FRAP measurements and silicon surfaces allows the discrimination of cell membrane portions and sets the stage for study of the dynamic behaviour of functional cellular membrane markers.

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Mys, a family of mammalian transposable elements isolated by phylogenetic screening

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It has recently been demonstrated both empirically¹⁻³ and mathematically⁴ that transposable elements may spread rapidly throughout a population once introduced even when they dramatically reduce the fitness of individuals that carry them. Such events result in pronounced differences in the phylogenetic distribution of genetic elements capable of rapid genome invasion. Using a simple and general procedure to screen the genome of the white-footed mouse *Peromyscus leucopus*, we have isolated a family of retrovirus-like elements which is apparently absent from the genome of the house mouse *Mus domesticus*. Here, we report this procedure and an analysis of the organization, phylogenetic distribution and sequence of this family of transposable elements.

We have devised a simple procedure designated ϕ -screen (ϕ for phylogenetic) to search for elements that classical genetic experiments⁵ had suggested were present in the genome of *P. leucopus*. The ϕ -screen is based on the assumption that rapid invasion of the genome of a species by a family of transposable elements results in distinct phylogenetic DNA differences from other related species, and that such differences persist in descendant species. A recombinant library of *P. leucopus* DNA was constructed and 60 randomly chosen clones from this library were used in a ϕ -screen against *M. domesticus* DNA (Fig. 1a). The procedure involves testing the repetitive sequences in these clones for their relative abundances in *P. leucopus* DNA and in the DNA of a related species to identify sequences that show major differences. Because the rate of acquisition of new DNA families is probably not constant across all species, the choice of the best phylogenetic distance for a species to screen against may involve some trial and error. Four clones showed major differences in hybridization patterns in this ϕ -screen, and a detailed characterization of one of these clones is presented here.

Figure 1a shows a pair of duplicate filters hybridized to the total genomic *P. leucopus* and *M. domesticus* probes. In lane 2, restriction segments giving significant hybridization with the *P. leucopus* probe are not hybridizing detectably with the *M. domesticus* probe, suggesting that these sequences are abundant in the *P. leucopus* genome but not in the *M. domesticus* genome. We call this repetitive sequence family *mys*, from the Greek word for mouse. When the DNA carried in this clone was restriction

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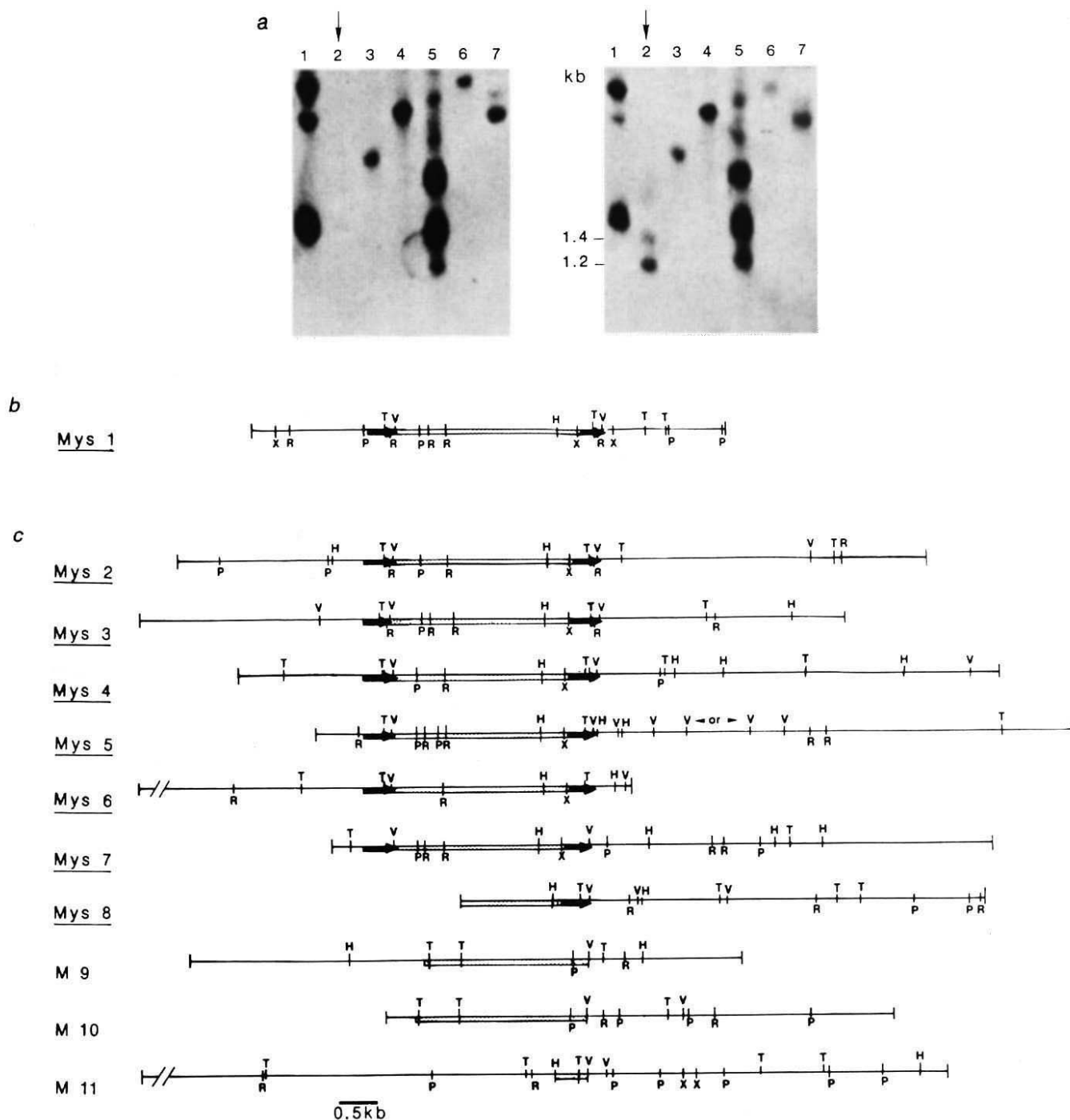


Fig. 1 ϕ -Screen identifying a repetitive DNA sequence which shows differences in phylogenetic distribution, and restriction maps of various members of the *mys* element family. **a**, ϕ -Screen autoradiograms of 7 *P. leucopus* library clones showing bands containing repetitive DNA sequences. Randomly selected cloned DNAs were prepared²⁰ and cleaved with a combination of *EcoRI*, *BamHI* and *HindIII* to facilitate the separation of highly repeated sequences, which would drown out any differential hybridization resulting from the less repeated sequences of interest. The restriction digests are then electrophoresed through duplicate 0.8% agarose gels and Southern blotted²¹ to nitrocellulose. One blot is hybridized to a radioactive genomic probe DNA from *P. leucopus* (right panel) and the duplicate blot is hybridized to a genomic probe from *M. domesticus* (left panel). Hybridizations were at 60 °C as previously described²². Single-copy sequences do not give a detectable hybridization signal, while sequences that are relatively abundant give a hybridization signal that reflects their genomic repetition in the probe DNA. Despite the overall similarity of the two panels, lane 2 reveals two distinct bands of 1.2 and 1.4 kilobases (kb) which hybridize only to the *P. leucopus* probe. Observed differences between the two probes were confirmed by washing radioactivity from the filters with alkali and reversing the hybridization probes to guard against artefacts. The clones displayed in these panels are actually a collection of those showing the greatest variation, therefore the frequency of bands showing differences in hybridization intensity is greater than normally observed. **b**, Restriction enzyme map of the clone from lane 2 revealing the two clusters of *PstI*, *EcoRI* and *EcoRV* sites. The bar and arrows indicate the portion of the clone which shows detectable hybridization to *P. leucopus* DNA but not to *M. domesticus* DNA. **c**, Restriction enzyme maps of 10 other clones that were isolated from the *P. leucopus* library using the 2.5-kb *EcoRV* fragment of *mys-1* (**b**) as probe. Clones *mys-2* to *mys-7* appear to contain intact elements. The *mys-8* element seems to have suffered a deletion and has also been truncated in the cloning process. Clones M9 to M11 appear to be shortened, divergent versions of the *mys* element repetitive sequence. Dark arrows in **b** and **c** indicate the 343-bp direct terminal repeat. X, *XhoI*; R, *EcoRI*; P, *PvuII*; T, *PstI*; V, *EcoRV*; H, *HindIII*. *mys-2* contains two additional *PvuII* sites (not shown), which split its most 5' *PvuII* fragment into bands of approximately 1,150, 195 and 65 bp. *mys-5* also has one *EcoRV* site that has not been fixed (as shown).

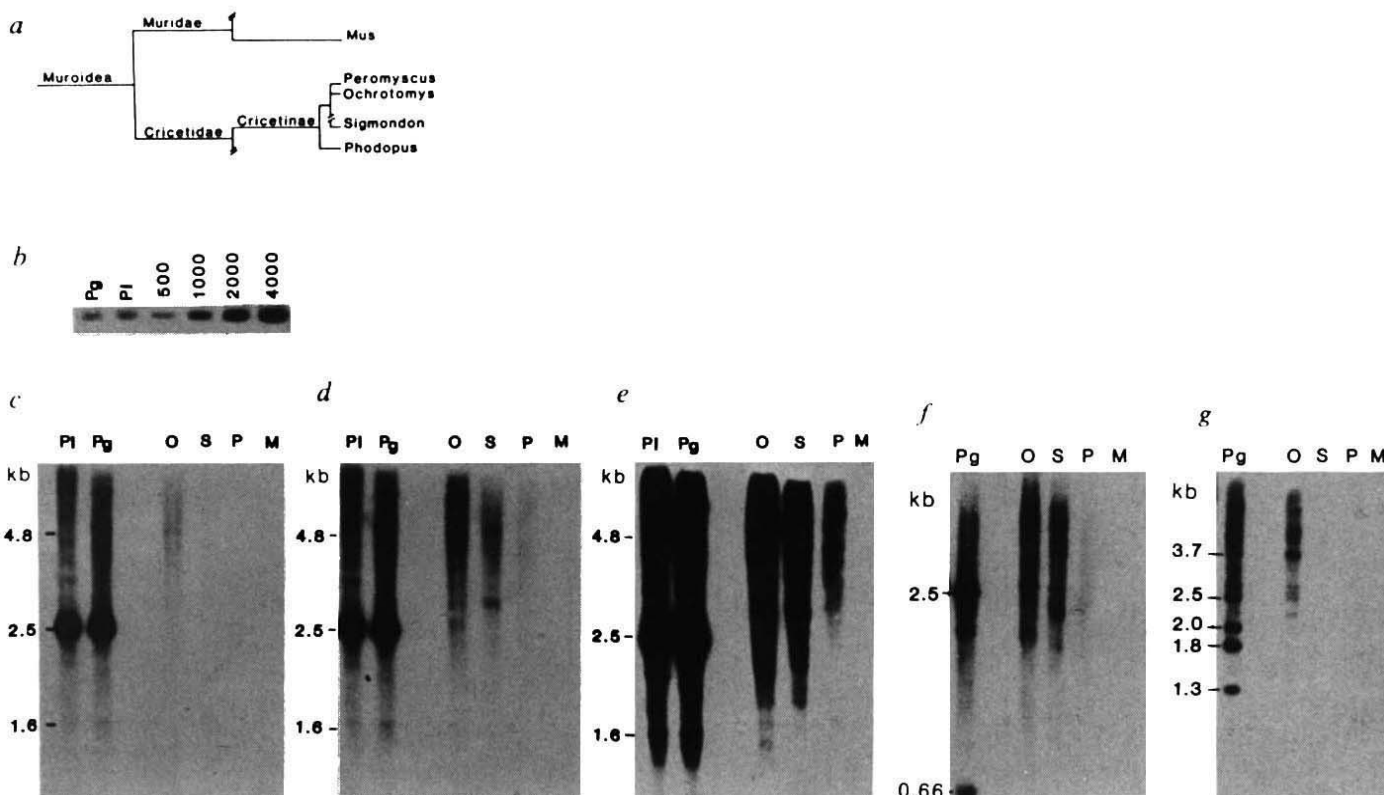


Fig. 2 Phylogenetic distribution, copy number and organization of the *mys* elements. **a**, Evolutionary tree depicting relationships of these species adapted from Eisenbert²³. Brownell²⁴ has used reassociation kinetic analyses of low copy sequences to estimate that the Cricetidae and Muridae diverged between 38.5 and 58 million years ago. **b**, In this experiment to determine copy number, *EcoRV* digests of *P. leucopus* (P1), *P. gossypinus* (Pg) and various genomic haploid equivalents of cloned *mys*-1 DNA were electrophoresed on 0.8% agarose gels, Southern blotted and hybridized to nick-translated probe consisting of the internal *EcoRI*-*HindIII* segment of *mys*-1. **c**, **d** and **e** are similar Southern blots of *EcoRV*-digested DNAs from *P. leucopus* (P1) mouse and its sibling species *P. gossypinus* (Pg), the golden mouse, *Ochrotomys* (O), the cotton rat *Sigmondon* (S), the hamster *Phodopus* (P), and the house mouse *Mus* (M), again electrophoresed on 0.8% agarose gels and hybridized to radiolabelled probe from the internal *EcoRI*-*HindIII* segment of the cloned *mys*-1 element. In **c**, the hybridization was at 60 °C, whereas in **d** and **e** a lower stringency 50 °C hybridization was used. The **c** and **d** hybridizations included internal plasmid copy number control lanes as per **b** (not shown), and were exposed so that these were equivalently intense. Therefore the increased hybridization in **d**, for example in lanes O and S, reflects better detection of more divergent copies of *mys* elements, and eliminates the possibility that these species simply carry a lower copy number of elements identical to *mys*-1. Panel **e** is simply a longer exposure of **d** to illustrate the apparent absence of sequences with detectable homology in *Mus* (M). DNAs were also digested with *PstI* (**f**) and *EcoRI* (**g**), electrophoresed through 0.8% agarose gels, blotted to nitrocellulose and hybridized at 50 °C to the same probe. The major band in both the *EcoRV* and *PstI* digests is of the predicted 2.5-kb size, but *PstI* also yields a second major band of 0.66 kb which is not predicted by any of the mapped *mys* clones. The major band in the *EcoRI* digest is the 1.8-kb band predicted by the *mys*-1 structure, but three other prominent bands also occur. Two of these, the 2.0- and 2.5-kb bands, are consistent with loss of the second, and the first and second, internal *EcoRI* sites respectively, while the third band of 1.3 kb suggests that an additional internal *EcoRI* site frequently occurs. Heterogeneity at the first internal *EcoRI* site is in fact notable among the mapped *mys* elements in Fig. 1c.

mapped (Fig. 1b), two clusters of *PstI*, *EcoRI* and *EcoRV* restriction sites were found to flank the region of differential hybridization, suggesting the presence of direct repeats. Ten other clones with homology to *mys*-1 were isolated from the *P. leucopus* library (Fig. 1c). Most members of the *mys* family are quite similar, with restriction patterns showing significant homology, but no two elements are exactly alike. In each case except *mys*-6 the terminal repeats of a single element show identical patterns, even though there is often variation between the terminal repeats of one element and those of another. This suggests that there is a single copy of most of the terminal repeat during transposition which is then duplicated to give the complete element, as occurs during reverse transcription of retroviral genomes⁶.

To further investigate the organization and phylogenetic distribution of *mys*-homologous sequences within the superfamily Muroidea, Southern-blot hybridization studies were done on DNA from *Mus*, a Muridae, and five species of Cricetidae. The phylogenetic relationship of these species is shown in Fig. 2a. Figure 2b-g demonstrates that most *mys* elements in both *P. leucopus* and its sibling species *Peromyscus gossypinus* are of the same size class and general sequence organization as *mys*-1. An experiment designed to count only these structurally con-

ved elements, shown in Fig. 2b, indicates that there are between 500 and 1,000 copies per haploid genome. Although the major bands are remarkably consistent between these sibling species of *Peromyscus*, minor band differences suggest that the *mys* family may be somewhat variable even between these closely related species. *Ochrotomys*, *Sigmondon* and *Phodopus* all have repetitive sequences homologous with *mys*, although hybridization is best observed to *Sigmondon* and *Phodopus* DNA at lower stringency (Fig. 2d, e). Banding patterns differ between these species, suggesting that the structure of *mys*-homologous sequences is not conserved throughout the Cricetidae. No hybridization to *Mus* DNA was detected even at lowered stringency.

The complete nucleotide sequence of *mys*-1 is shown in Fig. 3. The element does carry direct terminal repeats as suggested by the restriction map. These are 343 base pairs (bp) long and differ by single base changes at two positions. The element is flanked by a 6-bp direct repeat (Fig. 4) which presumably represents a target site duplication. There are three regions of significantly long open reading frame (ORF), a 489-bp uninterrupted sequence, and two adjacent regions making a 642-bp stretch interrupted by one stop codon.

Three striking features of the sequence indicate that the *mys*



Fig. 3 Complete sequence of *mys-1*. **a**, The sequencing strategy. DNA was 3'-end labelled with Klenow fragment and sequenced according to the procedure of Maxam and Gilbert²⁵. **b**, The underlined sequences are the 343-bp direct terminal repeats. The overlined sequences are regions of ORF. The pyrimidine tract, tRNA binding sequence, T₁₄A₃ sequences and internal direct repeats (IDRs) are boxed and labelled. Two L-shaped arrows delineate the region of ORF that is translated and analysed in **c**. The two bases of mismatch between the direct terminal repeats, and the two bases of mismatch between the internal direct repeats are identified by single base boxes. **c**, Comparison of amino acids found in reverse transcriptase (RT) and putative RT proteins. Boxed letters represent amino acids in *mys-1* with homology to at least two of the other sequences shown. Periods indicate gaps introduced to maximize homology. Filled triangles mark amino acids within the displayed region previously found to be invariant⁸. An arrow shows the location of the one amino acid common to the top four RT sequences shown, but absent in *mys-1*. The highly conserved RT sequence YXDD, which is normally located must 3' to the region shown, is absent in all three frames of *mys-1*, thus discounting a simple frame shift mutation at this point. Significant homology of *mys-1* with other previously identified RT regions was not found^{8,9}. The sequence data for Moloney murine leukaemia virus (MoMuLu) Rous sarcoma virus (RSV), cauliflower mosaic virus (CaMV) and 17.6 was obtained from refs 26, 27, 29 and 9, respectively.

transposable elements are closely related to retroviruses and probably transpose by a reverse transcription mechanism. First, at the internal junction with one long terminal repeat (LTR), where retroviruses commonly carry a stretch of polypurines on one strand of DNA, and, of course, polypyrimidines on the other, we find an uninterrupted string of 21 pyrimidines. Second, at the other internal junction where retroviruses have a sequence that binds a transfer RNA to serve as a primer during reverse transcription, we find 12 bases that perfectly match the 3' end of a mammalian lysine transfer RNA⁷. Finally, translation of the 489-bp ORF from *mys-1* reveals many amino-acid homologies with conserved regions of four other reverse transcriptase proteins (Fig. 3c)^{8,9}. Despite these similarities, the *mys* elements are distinguished from retroviruses both by their small size and by their unusual sequence organization, with the coding sequence on the strand opposite the normal retroviral configuration relative to the polypurine tract and tRNA binding sequence.

In Fig. 4 the sequences of the repeats of *mys-2* are compared

with each other and with the direct repeats of *mys-1*. Although the 6-bp target site duplications generated by *mys-1* and *mys-2* showed no apparent similarity, we observed a striking homology between the sequences 3' of these duplications, where 12 out of 15 bases are identical, and 10 bases further downstream we observe another 6-bp identity (Fig. 4). This strongly suggests that these elements exhibit target-site specificity during movement. The unexpected sequence complexity of the target site allows the speculation that the copy number of *mys* elements may simply be limited by the number of available target-site sequences in the genome.

To understand better the nature of the 489-bp ORF observed in *mys-1*, we sequenced the corresponding region of *mys-2* (data not shown). Multiple single-base changes and frameshift mutations were found that eliminated the long ORF. This portion of a *mys* element is, therefore, not always encoding protein, and the ORF of *mys-1* may itself be a shortened, divergent version of a functional coding sequence that might be carried on only a few if any of these elements.


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Mys 2 left  CCGTCTTTTAAAGAAAGGCTTGTTCGTCGAGGGCTTTTCTCCAGGTTCCCAAGCCCGGAGTCCCAATCCACTTATAAAATCACTCAGACGCTT 80
Mys 2 right TGTTCGTCGAGGGCTTTTCTCCAGGTTCCCAAGCCCGGAGTCCCAATCCACTTATAAAATCACTCAGACGCTT
Mys 1 left  TCCCTTCATGCCAAGGCTCTT.....C.....T.....
Mys 1 right .....C.....
Mys 2 left  ATATCACTTATAAACTGTATGAGTGGCAGGGCTTCTGCTAACTGCTCTTTTATCTTAAATTAAGCCATTTCTATAATCTATACCTGCCACGTGGCT 180
Mys 2 right ATATCACTTATAAACTGTATGAGTGGCAGGGCTTCTGCTAACTGCTCTTTTATCTTAAATTAAGCCATTTCTATAATCTATACCTGCCACGTGGCT
Mys 1 left .....G.C.....T.....G.....
Mys 1 right .....G.CA.....T.....
Mys 2 left  GGTGGCTTACCAGAGTCTCTACATGCTGCTTCTCTCGGGTGGCTGCACTCTCTCCCTCAGCGCTTCCGCTTCCCAAGTTCCTCTCTCTCTGCTCCA 280
Mys 2 right GGTGGCTTACCAGAGTCTCTACATGCTGCTTCTCTCGGGTGGCTGCACTCTCTCCCTCAGCGCTTCCGCTTCCCAAGTTCCTCTCTCTCTGCTCCA
Mys 1 left .....G.C.....G.....TT.....C.....
Mys 1 right .....G.C.....G.....TT.....C.....
Mys 2 left  CCTACCTCCTGCCTGCTCATTGGCCATCAGTGTATTTATTTACATAGAGTATATCCACAGCAAGGGTTCATCCCATTAAGTTCTGGATGGTAATAAAGA
Mys 2 right CCTACCTCCTGCCTGCTCATTGGCCATCAGTGTATTTATTTACATAGAGTATATCCACAGCAAGGGTTCATCCCATTAAGTTCTGGATGGTAATAAAGA
Mys 1 left .....GGCTCTATCCCTCTTAACTTGTCTACTAGATAAAG
Mys 1 right .....GGCTCTATCCCTCTTAACTTGTCTACTAGATAAAG

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Fig. 4 Flanking sequences and direct terminal repeats of *mys-1* and *mys-2*. The first base of each terminal repeat, shown in register, is marked by the number 1. Periods mark positions where the *mys-1* sequence is the same as for *mys-2*. Dashes indicate deletions and the circumflex indicates that the *mys-1* repeats carry a TT insertion relative to *mys-2* at that position. The terminal repeats of *mys-2* differ at only two positions by a single base change. These repeats are not, however, different from each other at the same positions as are the terminal repeats of *mys-1*. *mys-1* and *mys-2* repeats differ from one another at multiple positions, confirming the observed restriction site heterogeneity. The boxes of six bases flanking the terminal repeats represent the target site duplications. Various flanking sequence identities indicating the possible target site sequence, are marked correspondingly with overlines and underlines.

We have performed comparisons of the *mys-1* sequence by computer analysis using the Stanford SEQ program and sequence data from GenBank. A comparison with the murine *IAP* family¹⁰⁻¹² revealed only one significant homology between *mys* and available sequenced portions of *IAPs*. This 76.5% homologous region¹³ included the polypyrimidine tract and extended 9 bp into the *mys* LTR, covering a total of 32 bp. In light of previous homologies found between diverse retroviruses and transposable elements, like the 10-bp identity at the ends of the *Drosophila copia* and yeast *Ty1* elements¹⁴, this degree of homology probably does not indicate a close relationship between the *mys* and *IAP* families. This is compatible with the observation that they use different tRNA binding sequences, and with the apparent absence of *mys* sequences in *M. domesticus*, where *IAP* elements are abundant. In comparing *mys* sequences with other characterized murine repetitive elements such as *LTR-IS*^{15,16}, *VL30*¹⁷, *MS3*¹⁸ and with the entire GenBank viral and mouse data files, we found no significant homology.

The structural features of *mys-1*, and the fact that these sequences are abundant in *Peromyscus* and closely related cretoid rodents but apparently absent in *Mus*, suggested to us that this might represent a novel family of mammalian retrotransposons. Although the structural similarities between these elements and the retroviruses may reflect their common descent from early transposable elements, it is also quite plausible that many of the extant transposable elements evolved directly from retroviruses¹⁹. This second possibility would certainly explain the sudden appearance of new mobile sequences in a phylogenetic lineage.

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Transcription, processing and nuclear transport of a B1 *Alu* RNA species complementary to an intron of the murine α -fetoprotein gene

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The *Alu* sequence family comprises the major dispersed repeat sequences of rodent and primate genomes, numbering >300,000 copies in the human haploid genome¹⁻³. The function of these elements is unknown². The sequences can be transcribed by RNA polymerase III and represent a substantial fraction of total heterogeneous nuclear RNA²⁻⁷. *Alu* sequences can be found both in the flanking regions and within the transcription units of several well-characterized genes. Here we show that some members of the mouse B1 *Alu* sequence family encode a small cytoplasmic RNA. The mouse B1 sequence is $\approx$ 130 nucleotides long and shows homology with the monomeric units of the dimeric 300-nucleotide primate sequence⁸. By means of microinjection studies in the *Xenopus laevis* oocyte, we have elucidated a novel pathway leading to the appearance of a processed B1-type *Alu* RNA species in the cytoplasm. The abundance of this small *Alu* RNA differs between various mouse tissues, suggesting a role in tissue-specific gene expression.

Here we studied the *Alu* sequence contained within the first intron of the mouse α -fetoprotein (α -FP) 'minigene' (see Fig. 1 for the description of this gene)⁹. The transcript of this sequence is complementary to the first intron of the pre- α -FP messenger RNA species⁹.

On injection of the α -FP minigene into the nucleus of *X. laevis* oocytes, we observed the appearance of a 210-nucleotide (nt) transcript (Fig. 1a); this species corresponds to the 220-nt transcript generated on transfection of the α -FP minigene in monkey kidney cells⁹, initiating at the 5' end of the *Alu* sequence

Fig. 1 Transcription and processing of the B1 *Alu* sequence in *X. laevis* oocytes. Plasmid DNA was transcribed in an intact *X. laevis* oocyte following gene microinjection²¹. RNA was labelled with [α -³²P]GTP, extracted, and analysed by electrophoresis in a 10% acrylamide gel under denaturing conditions as described previously^{21,22}. **a**, RNA transcripts generated in the oocyte. Lanes 1–3, products after 45 min, 1 h and 2 h, respectively. **b**, Effect of α -amanitin on *Alu* sequence transcription in the intact oocyte. The gene was co-injected in a volume of 20 nl with either buffer alone or α -amanitin, into the germinal vesicle to yield an intranuclear concentration as indicated: lane 1, no α -amanitin; lanes 2–5, 0.1, 1, 10 and 100 μ g ml⁻¹, respectively. **c**, Precursor-product relationship of the 210- and 135-nt RNA. The ³²P-labelled 210-nt primary transcript was synthesized in a KB cell-free system¹¹. RNA was injected into the germinal vesicle of *X. laevis* oocytes, and maintained at 21 °C in OR-2 buffer²¹ for the stated times. RNA was extracted and analysed by electrophoresis as described for **a**. M is a ³²P-labelled *Hinc*I digest of ϕ X174. **d**, Schematic diagram of the mouse α -FP minigene. The figure is based on the sequence provided by Young *et al.*⁹. The α -FP minigene is contained on plasmid pAFZE. Δ id⁹, given by Dr Shirley Tilghman. Heavy lines denote the sense strands of either the α -FP gene or the *Alu* sequence, with arrows indicating the transcriptional polarity (5' to 3'). E and I denote exon and intron sequences, respectively. The *Alu* sequence is presented, with I, P and T denoting the sites of transcription initiation, processing and termination determined in this study. The direct repeats are boxed.

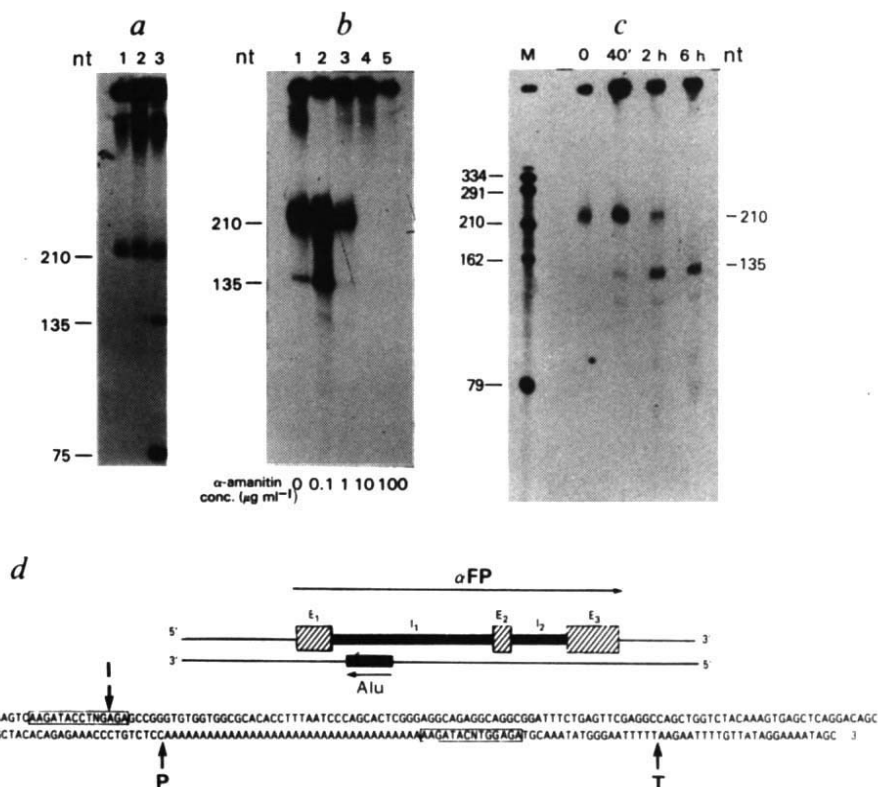
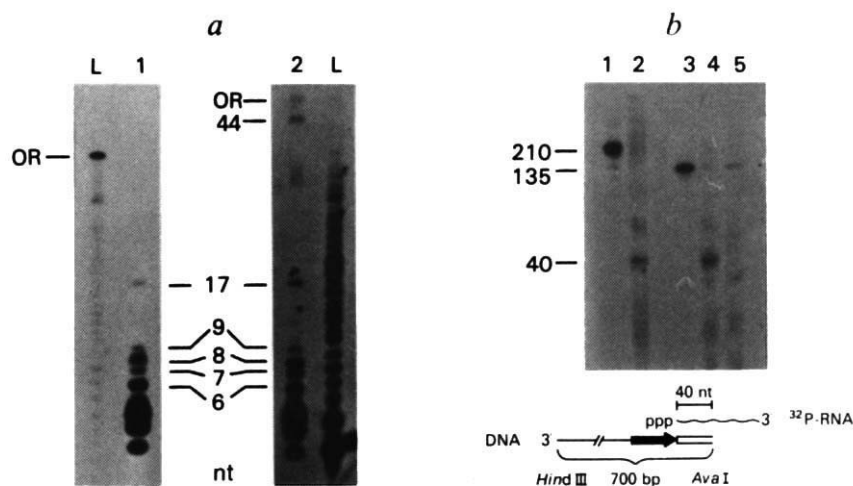


Fig. 2 Determination of the processing site within the 210-nt species. **a**, RNase T1 digest of the 210- and 135-nt species. ³²P-labelled species were synthesized in the oocyte using [α -³²P]GTP and gel-purified. The RNA species were digested with RNase T1 (ref. 11) and analysed on a 20% sequencing gel. Lane 1, T1 digest of 135-nt species; lane 2, digest of the 210-nt species. L, partial acid hydrolysate of ³²P-5'-labelled yeast tRNA^{Phe}. **b**, Mapping the 5' ends of the 210- and 135-nt species by a 'reverse' S₁ nuclease analysis. ³²P-labelled RNAs were generated in the oocyte as described above and annealed to a 700-base pair (bp) fragment extending ~660 nt 5' to the putative start site of transcription in the *Alu* sequence (with respect to the *Alu* sense strand) and complementary to the first 40 nt of the putative 5' end of the *Alu* transcript. The hybridization mixture was digested⁹ and analysed on a 15% sequencing gel as described elsewhere⁹. Lanes 1 and 3, 210- and 135-nt species, respectively. Lanes 2 and 4, S₁ nuclease digestion products of the hybrid formed between the *Hind*III–*Ava*I fragment and the 210- and 135-nt species, respectively. Lane 5, S₁ nuclease digest of the 135-nt species in the absence of the *Hind*III–*Ava*I DNA fragment. Inset, S₁ nuclease mapping strategy. The heavy arrow denotes the direct repeat preceding the *Alu* sequence.



and terminating within the first run of five T residues beyond the 3' direct repeat (Fig. 1d). At 45 min after injection, this was the principal species (Fig. 1a, lane 1). On further incubation, both a 135- and a 75-nt species were also detected (Fig. 1a, lanes 2, 3). The sizes of these smaller species and the kinetics of their appearance suggested that they might represent the products of processing of the 210-nt primary transcript. At various time periods after 2 h the 75-nt species appears as a minor species relative to the 135-nt RNA, suggesting that it is relatively labile.

To demonstrate that the 210-nt species is transcribed by RNA polymerase III, we co-injected α -amanitin with the α -FP minigene at concentrations sufficient to inhibit transcription by this polymerase¹⁰. Only at high concentrations of α -amanitin (10 and 100 μ g ml⁻¹) was the appearance of both the 210- and 135-nt species inhibited (Fig. 1b, lane 4 and 5).

To demonstrate directly a precursor-product relationship between the 210-nt primary transcript and the 135-nt species, the ³²P-labelled primary 210-nt transcript was synthesized *in vitro* in a KB cell-free transcription system¹¹, then injected as a purified RNA into the nucleus of intact *X. laevis* oocytes. We observed that the 210-nt species disappeared with time, with the coincident appearance of the 135-nt species (Fig. 1c).

The nature of the processing reaction was determined by several methods. RNase T1 digestion of the 210- and 135-nt RNA species demonstrated that whereas both yielded the characteristic 17-nt 5' fragment arising 17 nucleotides from the 5' end of the *Alu* sequence, only the 210-nt RNA yielded the characteristic 44-nt 3' fragment (TCTCCA₃₈G) (Fig. 2a). The 5' termini of the 210- and 135-nt species were shown to be identical by S₁ nuclease mapping (Fig. 2b), with the site of transcription

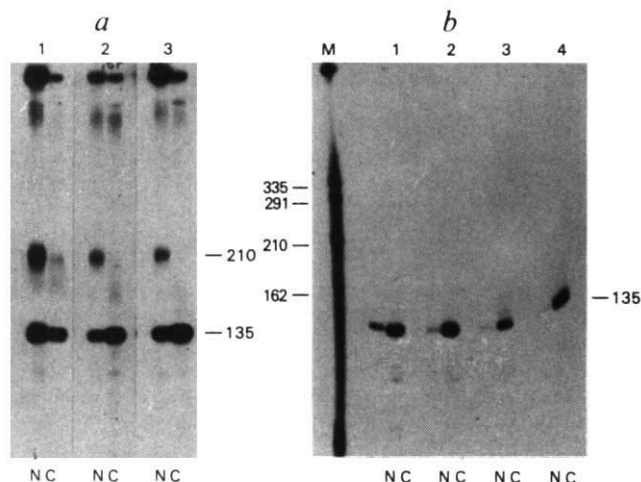
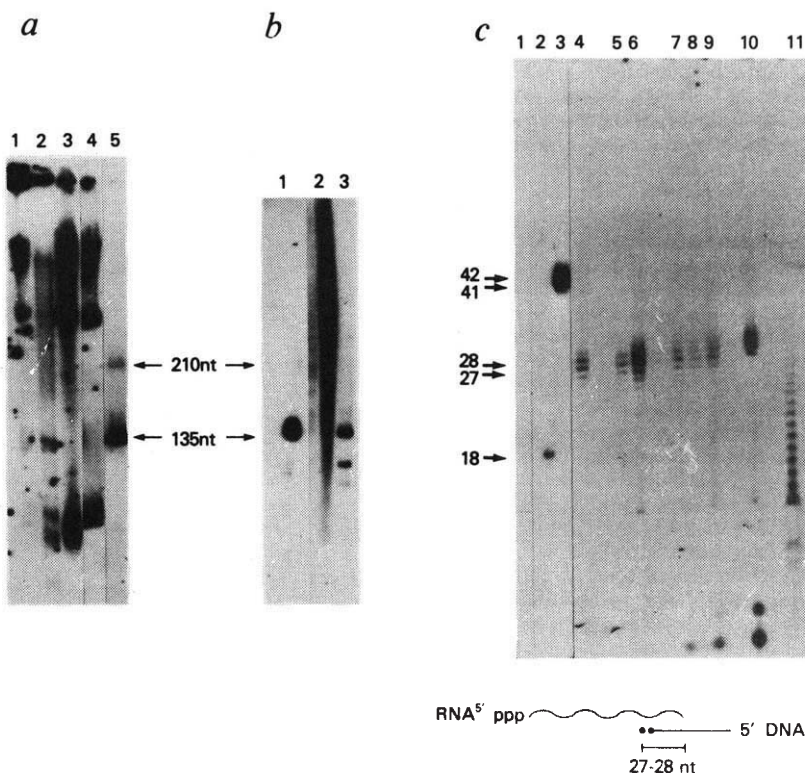


Fig. 3 Intracellular localization of the primary *Alu* transcript and the 135-nt processed species. RNA species were extracted from nuclear or cytoplasmic fractions of manually dissected oocytes using the trichloroacetic acid (TCA) fixation technique described previously²³. *a*, Distribution of species after injection of the α -FP gene and [α -³²P]GTP. Lanes 1–3, analysis performed after 4, 12 and 24 h, respectively. *b*, Distribution of the 135-nt species after direct introduction into the oocyte nucleus. The ³²P-labelled RNA shown in *a* was extracted from the gels presented and re-injected into the nucleus of intact *X. laevis* oocytes. Oocytes were manually dissected after TCA fixation at 1 h (lane 1), 4 h (lane 2), 12 h (lane 3) and 24 h (lane 4) and the RNA species present in either nucleus (N) or cytoplasm (C) were determined.

Fig. 4 RNA species homologous to the α FP B1 *Alu* sequence can be detected in mouse tissues.

Total RNAs from mouse tissues and *X. laevis* oocytes were isolated as follows: tissues and oocytes were homogenized in TMN buffer²²; SDS and proteinase K were added to 2% and 0.5 mg ml⁻¹, respectively, and the reactions were incubated for 30 min at 37 °C. The suspensions were extracted twice with phenol-chloroform (1:1) and ethanol-precipitated. The precipitates were resuspended in 400 μ l of DNase buffer (50 mM Tris-HCl pH 7.5, 5 mM MgCl₂), 50 μ g ml⁻¹ DNase I (RNase-free, Miles) was added and the reactions incubated for 15 min at 37 °C. The reactions were stopped by adding SDS and proteinase K, extracted with phenol-chloroform and the RNA precipitated with ethanol. *a*, Northern analysis of: lane 1, total cellular RNA from adult liver; lane 2, fetal liver; lane 3, adult kidney; lane 4, adult brain; lane 5, total RNA extracted from *X. laevis* oocyte previously injected with the α FP minigene. 75 μ g of each total RNA fraction was electrophoresed on a 6% urea/acrylamide gel²² and electroblotted onto Gene Screen Plus membranes (NEN). A 2-kilobase (kb) *Bam*HI-*Eco*RI fragment containing the mouse *Alu* sequence was excised from the minigene⁹, gel-purified, nick-translated with [α -³²P]dCTP and used as probe. Identical results were obtained using the 0.7-kb *Sac*I-*Hind*III fragment comprising the first intron, the second exon and sequences of the second intron. The membrane was hybridized at 42 °C for 36 h in 50% formamide, 5 $\times$ SSC, 1 $\times$ Denhardt's, poly(rA) at 100 μ g ml⁻¹, and denatured calf thymus DNA at 100 μ g ml⁻¹. Blots were washed twice with 2 $\times$ SSC at 37 °C, twice with 2 $\times$ SSC, 1% SDS at 65 °C and twice with 0.1 $\times$ SSC at room temperature, then autoradiographed. *b*, Northern analysis of: lane 1, total RNA extracted from *X. laevis* oocytes injected previously with the α -FP minigene; lanes 2 and 3, nuclear and cytoplasmic RNA from Hepa-I cells. The smaller species of ~110 nucleotides in lane 3 is frequently seen on storage of the purified 135-nt species generated in the *X. laevis* oocyte and therefore may represent a degradation product. Nuclear and cytoplasmic RNAs were isolated²⁴ from Hepa-I cells, electrophoresed, and transferred to Gene-Screen Plus membranes as described for *a*. The blot was hybridized to a 5'-³²P-labelled 30-residue synthetic oligonucleotide (OCS Laboratories, Denton, Texas) complementary to nucleotides 75–104 of the α -FP *Alu* sequence (see Fig. 1). The blot was hybridized for 24 h at 28 °C in 10 ml of a solution containing 0.1 μ g ³²P-labelled probe (1 $\times$ 10⁸ c.p.m. μ g⁻¹), 50% formamide, 1% SDS, 1 $\times$ Denhardt's, 50 mM sodium phosphate pH 7.0, 5 $\times$ SSC, poly(rA) at 100 μ g ml⁻¹, and denatured *Escherichia coli* DNA at 250 μ g ml⁻¹. The blot was washed twice at 22 °C in 2 $\times$ SSC, then twice in the same buffer containing 1% SDS at 40 °C, dried, and autoradiographed. *c*, S₁ nuclease mapping of the 3' terminus of the B1 RNA species isolated from mouse cells. Lane 1, probe, digested with S₁ nuclease without added RNA; lane 2, ³²P-labelled 18-nt standard; lane 3, 3' end-labelled probe; 4, cytoplasmic RNA from Hepa-I cells (4 μ g). Lanes 5–9, total cellular RNA from: 5, adult liver (25 μ g); 6, fetal liver (25 μ g); 7, adult brain (25 μ g); 8, fetal brain (25 μ g); and 9, adult kidney (25 μ g). Lane 10, RNA extracted from oocytes injected with α -FP minigene. Lane 11, ³²P-labelled oligonucleotide sizing ladder. Inset, S₁-nuclease mapping strategy. RNA was isolated as described for *a* and *b*. A 3'-³²P-labelled probe was prepared by addition of 1–2 C residues to a 40-residue synthetic oligonucleotide (3'-CCGATGTGTCTCTTTGGGACAGAGG(T)₁₅) using [α -³²P]CTP (700 Ci mmol⁻¹) and deoxynucleotidyl transferase²⁵. Hybridization of the probe to the RNA was performed in a volume of 20 μ l containing various amounts of RNA as noted, 0.125 pmol ³²P-labelled probe (10⁸ c.p.m. μ g⁻¹), 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 0.4 M NaCl, 0.1% SDS. Hybridization was for 1 h at 60 °C. S₁ nuclease digestion followed standard procedures⁹. The hybridization samples were transferred to 0.25 ml of a solution containing 0.28 M NaCl, 0.05 M sodium acetate pH 4.6, 4.5 mM ZnSO₄, 2 μ g ml⁻¹ denatured *E. coli* DNA, and 200 units of S₁ nuclease (Sigma). After incubation at 22 °C for 1 h, the digestion was stopped by adding 75 μ l of a solution comprising 0.2 M ammonium acetate, 5 mM EDTA 10 μ g ml⁻¹ denatured *E. coli* DNA. Nucleic acids were then extracted and collected by ethanol precipitation at -70 °C following addition of 20 μ g of yeast tRNA and 2 vol. ethanol. Samples were analysed on a 15% acrylamide sequencing gel, and autoradiographed.



initiation mapped to the 5' end of the *Alu* sequence (see Fig. 1). Nucleotide sequence analysis of P1 nuclease digests of the 210- and 135-nt species synthesized in the presence of either [α -³²P]GTP or [α -³²P]ATP demonstrated that both species bear either ppA or pppA at the 5' terminus (data not shown).

RNase T1 digestion of the unstable 75-nt species generated

the predicted fragment pattern, consistent with its origin as the 3' 75-nt trailer of the 210-nt primary transcript (data not shown).

The data demonstrated that the 210-nt primary *Alu* transcript is processed endonucleolytically at the 3' end to yield a 135-nt RNA. The site of cleavage lies just 5' to the oligoadenylate tract at the 3' end of the *Alu* sequence (Fig. 1). The processing reaction

thus cleaves a conserved 'core' *Alu* sequence from a 3' trailer which includes the A-rich sequence.

The intracellular localization of the 210- and 135-nt RNA generated in the *X. laevis* oocyte was determined by microdissection (Fig. 3). At 4, 12 and 24 h the primary transcript remained confined to the nucleus, while the 135-nt RNA accumulated in the cytoplasm (Fig. 3a). Injection of the purified 135-nt RNA into the nucleus resulted in its efficient transport from the nucleus to the cytoplasm, where it could be recovered as a stable species 24 h after injection (Fig. 3b). From these data the primary transcript seems to be an intranuclear species whereas the processed species appears to be cytoplasmic. The *Alu* RNA is transported to the cytoplasm only after the 3' trailer has been cleaved. The processing step thus provides a potential post-transcriptional control over the abundance of this species in both nucleus and cytoplasm.

We wished to determine whether this processed 135-nt *Alu*-specific RNA species could be detected in mouse tissues. By Northern analysis of total cellular RNA from fetal liver, using as a probe a fragment of the α -FP gene containing the *Alu* sequence, we detected a discrete species of 135 nt (Fig. 4a, lane 2) identical in size to the species accumulating in the *X. laevis* oocyte after injection of the α -FP minigene (Fig. 4a, lane 5). The species was not clearly visualized in adult liver (Fig. 4a, lane 1), adult kidney (Fig. 4a, lane 3) or adult brain (Fig. 4a, lane 4). Northern analysis using a 30-residue oligonucleotide specific for the B1 sequence, having no significant homology to either 7S or 4.5S (ref. 12), yielded similar data (not shown). With this probe the 135-nt species was readily detected in cytoplasmic RNA from the mouse hepatoma line, Hepa-I¹³ (Fig. 4b, lane 3).

To define the 3' terminus of the *Alu* RNA species in mouse tissues, RNA was hybridized to a 3' end-labelled oligonucleotide, and treated with S₁ nuclease (Fig. 4c). This probe is complementary to the primary *Alu* transcript, spanning the processing site, and was fully protected by the primary transcript in our digestion conditions (data not shown). S₁ nuclease mapping of the 3' terminus of the *Alu* RNA generated in the oocyte revealed protection of 27- and 28-nt species (Fig. 4c, lane 10), the fragments expected to arise from an RNA terminating just before the run of A residues or including one A. An identical pattern of protected probe fragments was seen with cellular RNA from fetal liver (Fig. 4c, lane 6) and cytoplasmic RNA from Hepa-I cells (Fig. 4c, lane 4). By means of this S₁ mapping procedure, these RNA species were also detected in adult liver (lane 5), adult brain (lane 7), fetal brain (lane 8), and adult kidney (lane 9). Based on the relative intensities of the protected probe and the amounts of RNA assayed, this processed *Alu* species appears to be present in ~10-fold greater abundance in fetal liver and the Hepa-I line than in the other mouse tissues analysed.

Here we have demonstrated that a member of the mouse B1 class of the *Alu* sequence family encodes a discrete cellular RNA species. Comparable studies have been performed in our laboratory on other mouse, rat and human *Alu* sequences. Some human *Alu* and the rat type II sequences generate primary transcripts in the *X. laevis* oocyte that are processed to a cytoplasmic 'core' *Alu*-specific RNA of ~300 nt in the case of the human gene, and ~160 nt in the case of the rat type II sequence (S.A.J. and M.Z., unpublished). We suggest that the pathway described for the mouse B1 *Alu* sequence represents a more general pathway characterizing both monomeric and dimeric *Alu* sequence transcripts.

Why are some *Alu* sequence transcripts, such as the mouse B1 sequence studied here, shuttled through the conversion pathway described? The efficient transcription of this B1 *Alu* unit, the precise processing of its primary transcript, the transport of the core *Alu* species from nucleus to cytoplasm, and the packaging of the processed B1 RNA into a ribonucleoprotein particle (S.A.-J. and M.Z., unpublished), suggests that this RNA has a role in either the expression of the gene in which it is located or some other process in fetal liver. One intriguing feature of

this B1 *Alu* sequence is that it encodes a natural antisense RNA of 210 nt complementary to the first intron of the α -FP pre-mRNA. Indeed, *Alu* sequences of the B1 and human type have been identified in the antisense orientation within several mouse^{14,15} and human^{16,17} genes. If the α -FP gene and the *Alu* sequence are being transcribed simultaneously, an RNA-RNA duplex of these transcripts could potentially form. From recent studies in both eukaryotic¹⁸ and prokaryotic^{19,20} systems, one might imagine that this primary *Alu* transcript would inhibit α -FP expression. The conversion pathway described here for the primary *Alu* sequence transcript suggests one mechanism by which this type of natural antisense RNA may be dealt with in a cell.

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An exonuclease protection assay reveals heat-shock element and TATA box DNA-binding proteins in crude nuclear extracts

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The ability to identify and purify *trans*-acting cellular factors that regulate eukaryotic genes is limited by the lack of a practical general assay. Current procedures using crude whole cell or nuclear extracts that restore transcriptional function *in vitro* or permit reconstruction of native chromatin at control sequences are effective only in select systems. I now present an exonuclease protection assay that is generally applicable for detecting sequence-specific DNA-binding proteins. The assay extends earlier work on the binding to the *Drosophila* heat-shock gene control element of a protein factor (HAP) present in crude nuclear extracts; the binding was shown by reconstitution of specific exonuclease resistance within a nuclease-hypersensitive site in chromatin^{1,2}. We show here that this same exonuclease resistance can be reconstituted on free linear DNA, despite many nonspecific binding activities present in unfractionated nuclear extracts. We have further applied this assay method to fractionate the protein factor that is bound constitutively to the heat-shock gene TATA box region in native chromatin¹. Exonuclease protection offers a sensitive, precise and rapid assay for any sequence-specific DNA-binding protein.

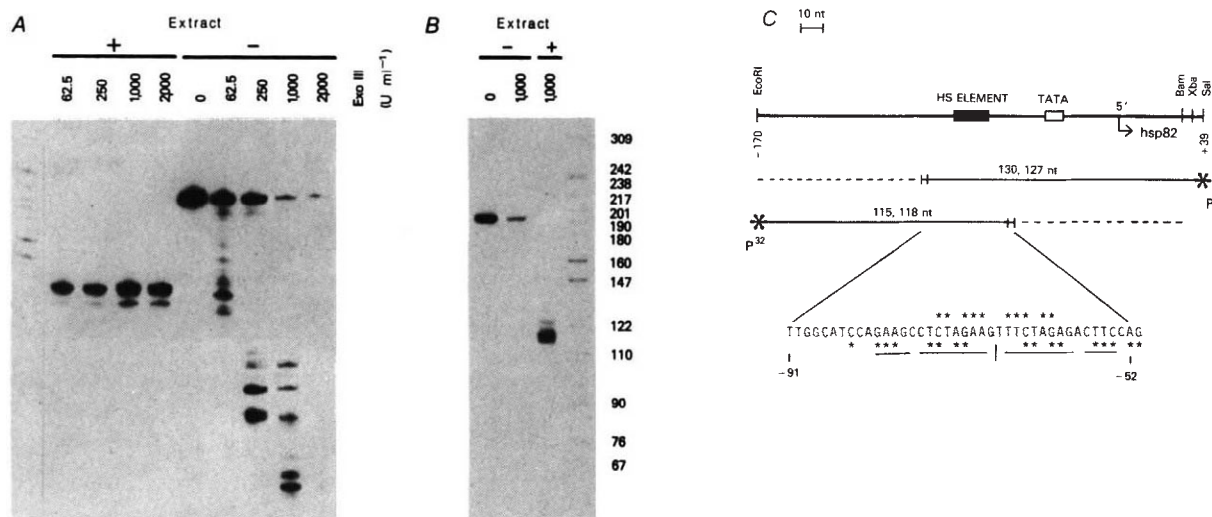


Fig. 1 ExoIII protection at the *hsp82* heat-shock control element conferred by nuclear extracts of heat-shocked *Drosophila* cells. A DNA fragment containing the *hsp82* gene 5' region was prepared from plasmid DNA (which consists of a *EcoRI* (–166)/*RsaI* (+20) fragment inserted into a pUC12 vector at the *EcoRI* and *SmaI* sites) by cutting at the restriction site chosen to be labelled followed by removal of the 5' phosphate with calf intestinal phosphatase, and by 5' end-labelling with polynucleotide kinase and [γ - 32 P]ATP according to standard procedures. The DNA, labelled at either the *SmaI* site (A) or the *EcoRI* site (B), as shown schematically in (C), was cut at the appropriate secondary restriction site to liberate the *hsp82* promoter fragment, which was isolated by polyacrylamide gel electrophoresis and by electroelution of the gel slice. For each binding reaction, 1,000 Cerenkov counts representing ~ 0.3 ng of DNA were incubated for 20 min (now amended to 15 min) at 24 °C in 50 μ l of binding buffer (75 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EGTA, 15 mM Tris-HCl pH 7.4, 0.5 mM dithiothreitol (DTT), 2 mM Na phosphate pH 7.0, 5% glycerol) containing 1 μ g ϕ X174 DNA cut with *HaeIII*, 10 μ g yeast tRNA, 1 μ g mixed deoxynucleotide (p(dN)₅, from Pharmacia-PL) and ~ 25 μ g of nuclear protein from the equivalent of $1\text{--}2 \times 10^6$ cells) extracted from heat-shocked Schneider line 2 cells essentially as described previously². All components were mixed in a 1.5-ml microfuge tube by gentle vortex. We have modified the former nuclear protein extraction procedure such that the crude, loosely packed nuclei are now extracted with 2.5 volumes of solution II² without re-adjustment of the final NaCl concentration; and the 100,000g supernatant is dialysed in standard dialysis tubing in solution III². A typical extract of 10^{10} cells yields 50–100 mg protein in 5–10 ml volume. The preparation of extract can be suitably scaled down several hundredfold to accommodate small quantities of cells (E. Zimarino, unpublished results). (Note that free histones bind strongly to DNA and block ExoIII movement; as such, it would be inadvisable to substitute a high salt extract of whole cells for the nuclear extract.) After the binding step, ExoIII (Boehringer-Mannheim) was then added as indicated to the protein-DNA complex (or the DNA alone, for the control experiment), mixed by gentle vortex and allowed to digest at 30 °C for 10 min. The reaction was terminated by the addition of an equal volume (50 μ l) of 20 mM Na_2EDTA , 1% SDS. The DNA was purified by organic extraction twice, precipitated in ethanol, redissolved in several microlitres of formamide/EDTA/dye, boiled for 2 min, quick-cooled in ice-water and electrophoresed on a short 5% sequencing gel according to standard procedures. 5' End-labelled marker DNAs (pBR322 cut with *HpaII*) were electrophoresed on flanking lanes of the gel. The gel was fixed briefly in 10% methanol, 10% acetic acid, dried and exposed to X-ray film with an intensifying screen at –70 °C, typically for an overnight exposure. In C, the exonuclease resistant strands due to protein binding which are visualized through the 5' label (asterisk) are aligned as solid lines below the map of the *hsp82* 5' region; the passage of ExoIII is indicated by dashes. The sequence of the region protected from convergent digestion is displayed; the stars denote nucleotides which match the heat-shock control element²⁴. Nucleotides which form the symmetrical dyad are underlined.

Protection of DNA from digestion by exonuclease, usually *Escherichia coli* exonuclease III (ExoIII)^{3–7}, locates the boundaries of protein-binding on DNA. This method has been used successfully to map the binding sites of several purified prokaryotic and eukaryotic DNA-binding proteins^{8–23}. The enzyme, a 3' to 5' exonuclease, degrades a double-stranded DNA fragment processively from both 3' termini until the substrate becomes single-stranded and resistant to further digestion. Specific protein binding blocks the passage of ExoIII and generates new fragments that remain resistant under limit digestion conditions. The ExoIII protection method is more sensitive than the standard DNase I protection (footprinting) technique as new exonuclease resistant fragments can be positively identified even if they occur as a small fraction of the total DNA population.

We were prompted to use the ExoIII protection method with crude rather than purified protein preparations because of the surprisingly clear demonstration of the binding to chromatin of HAP (heat-shock activator protein)^{1,2}; binding is shown by reconstitution of exonuclease resistance at nucleotide positions –50 to –86 in the *Drosophila hsp82* gene, following incubation of non-induced (embryonic) nuclei with 0.4 M NaCl extracts of heat-induced *Drosophila* cell nuclei² (+1 is the transcriptional start). ExoIII movement within the hypersensitive site in chromatin was not hindered by nonspecific binding proteins present in the unfractionated nuclear extract. Hence, we saw the possibility of detecting specific protein binding by exonuclease protection of free DNA incubated with crude nuclear

extracts; this procedure should in principle permit the identification of such proteins for any given DNA sequence.

Crude nuclear extracts contain, in addition to the DNA-binding activities of interest, endogenous nuclease and phosphatase activities that can destroy 32 P-labelled DNA fragments. We have found empirically that a mixture of Na phosphate and carrier nucleic acid consisting of transfer RNA, deoxyoligonucleotides and fragmented phage DNA can inhibit the endogenous nuclease activities sufficiently (but not totally) during the course of DNA binding and ExoIII digestion. Figure 1A shows the digestion pattern, at increasing concentrations of ExoIII, of a *EcoRI/SmaI* 209-base pair (bp) DNA fragment (Fig. 1C) containing 5'-flanking sequences of the *Drosophila hsp82* gene. When ~ 1 fmole of the fragment, 5' end-labelled at the *SmaI* site, is incubated with an extract of heat-shocked *Drosophila* cell nuclei, fragments of sizes 130 and 127 nucleotides (nt) (not well resolved from each other on this gel) are clearly resistant to ExoIII digestion at concentrations of the enzyme which reduce the DNA incubated without extract to small oligonucleotides. Increased ExoIII digestion (data not shown) does not eliminate the resistant fragments, which map the upstream boundary of the HAP binding site to position –91/–88. A similar experiment using the fragment 5' end-labelled at the *EcoRI* site maps the downstream boundary to position –52/–55 (Fig. 1B). These results are consistent with the mapped boundaries of HAP binding to chromatin (–50 to –86). HAP-binding activity to free DNA is abolished by

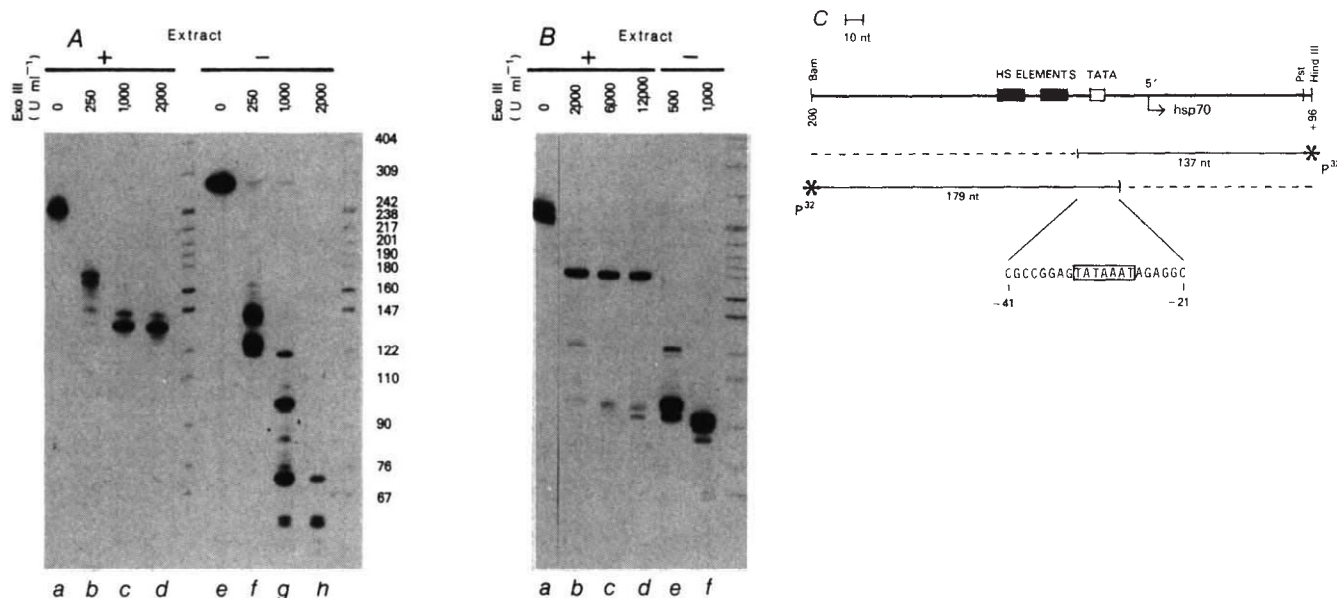


Fig. 2 ExoIII protection at the *hsp70* TATA box region conferred by nuclear extracts of non-shocked *Drosophila* cells. A DNA fragment containing the 5' region (*Xho*I (-194) to *Pst*I (+85)) of a *hsp70* gene from chromosomal locus 87C was 5' labelled at either end following procedures described in Fig. 1 legend. C shows a map of the fragment. Aliquots of 1,000 Cerenkov c.p.m. of the DNA labelled at the *Hind*III site (A) or at the *Bam*HI site (B) were incubated with 100 μ g of extract protein (or without extract protein) obtained from non-shocked Schneider line 2 cells, and digested with the indicated concentrations of ExoIII, according to the procedure described in Fig. 1 legend. The DNA was purified, electrophoresed on a 5% sequencing gel, fixed, dried and autoradiographed. The sequence of the region protected against convergent ExoIII digestion is displayed in C; the TATA motif is boxed.

digestion with Proteinase K (the protease is inactivated with PMSF subsequently) and is competed specifically with 5'-flanking DNA of the *hsp82* gene (data not shown), as is the binding to chromatin². Note that resistance to exonuclease is not observed at the TATA box region in the experiment shown in Fig. 1B; extracts of heat-shocked cell nuclei contain relatively low levels of the TATA-binding activity and the binding is inherently weak for *hsp82*, as observed previously in chromatin².

The sequence of the binding site for *hsp82* (see Fig. 1C) includes the heat-shock control element²⁴ (consensus CT_GAA__TTC_AG) as well as two similar elements that overlap it on either side. These sequences form a 28-bp symmetrical dyad (underlined) that is poorly represented in the rest of the heat-shock gene family. It is likely that these unique features of the *hsp82* sequence dictate the higher affinity of HAP for the *hsp82* gene in chromatin², this higher affinity resulting in the special ease of induction of the *hsp82* gene. DNA competition experiments in progress also indicate that the binding affinity of HAP to *hsp82* DNA is stronger than its affinity to DNAs for other heat-shock genes.

The presence in chromatin of non-histone protein bound to the heat-shock gene TATA box region in both non-induced and induced states precludes the detection of this protein *in vitro* through reconstitution of the binding to chromatin. Figure 2 shows reconstitution of the binding to free DNA. We used a *hsp70* gene rather than the *hsp82* gene as substrate for the TATA-binding protein; protein binding in chromatin to the *hsp70* gene TATA box was shown previously to be extremely tight¹. When a *Bam*HI/*Hind*III 296-bp DNA fragment containing 5'-flanking sequences of a *Drosophila hsp70* gene is end-labelled at the *Hind*III site, incubated with an extract of non-shocked *Drosophila* cell nuclei and digested with ExoIII, a major resistant fragment of 137 nucleotides is observed in the limit (Fig. 2A, lanes a-d). Such a resistant fragment is not observed during digestion of the DNA alone (Fig. 2A, lanes e-h). A similar experiment using DNA labelled at the *Bam*HI site (Fig. 2B) reveals a resistant fragment of 179 nucleotides which is not observed in the digestion of DNA alone; even 12,000 ml⁻¹ of ExoIII does not eliminate the resistant fragment (Fig. 2B, lane d). Treatment of the extract with proteinase K abolishes the specific resistance to ExoIII (data not shown). In

the incubations without added ExoIII, mild endogenous exonuclease activity can also be observed in the nuclear extract (Fig. 2A, compare lanes a and e; Fig. 2B, lane a). Note that as the nuclear extract used for this experiment was prepared from non-shocked cells, HAP binding to the *hsp70* heat-shock element is not observed in the experiment shown in Fig. 2A. When extracts are prepared from heat-shocked cells, ExoIII resistance at the *hsp70* heat-shock elements as well as at the TATA box region can be observed (data not shown).

The lengths of the resistant fragments map the borders of the binding site to positions -21 and -41 of this *hsp70* gene. These positions are consistent with those mapped previously in chromatin for the average of the five *hsp70* genes (-12 to -40)¹. The sequence of the 20-bp binding site (Fig. 2C) has at its centre the TATA box, a motif originally identified by Goldberg and Hogness which is present in many eukaryotic genes at about position -30. The TATA box is essential for accurate transcriptional starts *in vitro*²⁵. In the conalbumin promoter and in the adenovirus 'late' promoter, the TATA box has been shown to form a stable pre-initiation complex with a HeLa cell factor, in the absence of RNA polymerase II²⁶. It is likely that the activity we have identified by *in vitro* binding to the *hsp70* TATA box region belongs to this class of transcription factor. As the TATA-binding factor is the only (tightly) bound protein within the 5' nuclease-hypersensitive site of the *hsp70* gene in the non-induced state¹, its binding is also likely to be crucial for the establishment of the nuclease-hypersensitive chromatin structure which could be conditional for subsequent HAP binding. Deletion of sequences upstream of the *hsp70* TATA box do not affect the hypersensitivity, but deletion of the TATA box region does²⁷. The TATA box region of *Drosophila* heat-shock genes shares a homology (-43)CG_GCG_GAGTATAAAT(A/G)-C(A/C)GGCGC(-19) that is directly superimposable over the binding site (-41 to -21). We are testing the hypothesis that the factor which binds to the *hsp70* TATA box region can also bind to the same region in the other heat-shock genes, but not to other *Drosophila* genes. We conjecture that all TATA-binding functions are governed by a family of such proteins, each recognizing a unique consensus sequence surrounding the TATA core sequence.

Figure 3 shows that the exonuclease protection technique

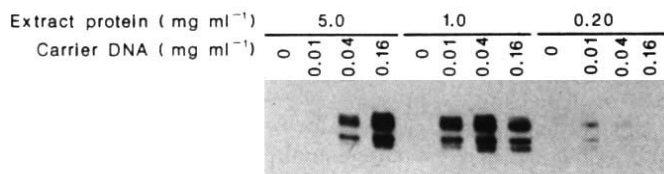


Fig. 3 Influence of extract protein and carrier DNA concentrations on specific ExoIII protection at the upstream border of the *hsp82* HAP-binding site. The *hsp82* gene fragment described in Fig. 1 legend was 5' end labelled at the *Sall* site and incubated with the indicated amounts of extract protein obtained from heat-shocked Schneider line 2 cells and Φ X174-*Hae*III-cut carrier DNA, and digested with 4,000 U ml⁻¹ of ExoIII. The resistant fragments were purified and electrophoresed as described above. (At such high concentrations of ExoIII, the minor sub-band below the major band(s) observed in Fig. 1A becomes more prominent).

used with crude nuclear extracts depends critically on the presence of carrier DNA, which preserves the specific DNA fragment during the binding and ExoIII digestion reactions (and presumably also competes away some of the nonspecific binding proteins). There is sufficient nuclease activity at extract protein concentrations of 5.0, 1.0 and 0.2 mg ml⁻¹, representing roughly 1/5th, 1/25th and 1/125th, respectively, of whole cell equivalent concentration, to remove essentially all of the ³²P label when the *hsp82* DNA fragment is incubated in the absence of carrier DNA. At each extract protein concentration, increasing amounts of added carrier DNA correspondingly increase the yield of the expected exonuclease-resistant fragments caused by HAP binding, although at high carrier DNA concentrations specific HAP-binding activity is lowered by significant competitive binding to carrier DNA. When the extract protein is diluted to 0.2 mg ml⁻¹ (1/125th of whole cell equivalent concentration), specific HAP-binding activity is reduced considerably; it is completely eliminated at a carrier DNA concentration of 0.16 mg ml⁻¹ (Fig. 3). In general, the optimal assay conditions for each individual DNA-binding protein depend on the relative activities in the extract of endogenous nuclease and phosphatase, and of the specific protein. Appropriate conditions should be determined by varying the concentrations of carrier DNA and of extract protein respectively. From the results in Fig. 3, the window of detectability is not prohibitively narrow. The other components we have used to preserve the labelled DNA give small improvements to the signal and are not absolutely necessary; other protective measures can also be substituted.

I have used the exonuclease protection technique to assay fractions obtained by column chromatography of the crude nuclear extract. HAP factor elutes from heparin-agarose with 0.6 M NaCl (Fig. 4A). When this fraction is chromatographed on a DNA-cellulose column, the binding activity elutes with 0.3 M NaCl; the combined purification is roughly 25-fold. Identical results are obtained with the more laborious chromatin-binding assay (data not shown). In an effort to purify HAP factor from mass cultures of heat-shocked *Drosophila* cells, we now routinely use exonuclease protection of free *hsp82* DNA for rapid assay of fractions obtained from chromatographic columns. The exonuclease protection procedure using free DNA requires only several hours' work, in contrast to the procedure using nuclear chromatin, which takes 5 days. We have also begun to purify the TATA-binding protein by column chromatography, using exonuclease protection of the *hsp70* DNA fragment as an assay. Figure 4B shows that the binding activity elutes from a heparin-agarose column with 0.6 M NaCl (and separates away from RNA polymerase activities; B. Walker, unpublished). Further chromatography separates this activity from HAP factor. As a test of its general applicability,

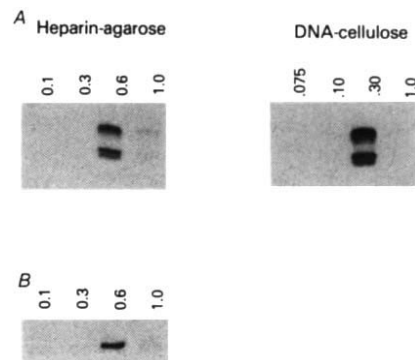


Fig. 4 Column chromatography of HAP and TATA-binding protein. **A**, HAP activity in heparin-agarose and DNA-cellulose column fractions was assayed by ExoIII protection of the *hsp82* gene fragment as shown in Fig. 1A. **B**, TATA-binding activity in heparin-agarose column fractions was assayed by ExoIII protection of the *hsp70* gene fragment as shown in Fig. 2B.

Methods. Minicolumns of 1 ml bed volume of heparin-agarose and DNA-cellulose (Pharmacia-PL) were equilibrated in chromatography buffer (CB)/0.1 M NaCl. CB consists of 20 mM Tris-HCl pH 7.9, 0.1 mM Na₂EDTA, 0.5 mM DTT, 10% glycerol, 0.5 mM phenylmethylsulphonyl fluoride. 1 ml (10 mg protein) of undiluted extract of heat-shocked cell nuclei was passed through heparin-agarose under gravity. The column was washed with 2.5 ml of CB/0.1 M NaCl, and step-eluted with 2.5 ml of CB/0.3 M, CB/0.6 M, and CB/1.0 M NaCl, respectively. Fractions were dialysed for 2 h in solution III², quick-frozen in liquid N₂ and stored at -70°C. The CB 0.6 M NaCl step fraction was then chromatographed on the 1-ml DNA-cellulose column, and the column was step-eluted with 2.5 ml of CB 0.075 M, CB/0.1 M, CB/0.3 M and CB/1.0 M NaCl, respectively. Fractions were dialysed, frozen and stored as above. Heparin-agarose fractions (3 µl) and DNA-cellulose fractions (5 µl) were assayed for HAP activity in the standard 50-µl reaction. 25 microliters of the heparin-agarose fractions were employed for assay of TATA-binding activity in the standard reaction.

the exonuclease protection technique is being used to identify sequence-specific binding proteins in a mammalian system.

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Subcellular distribution of oestrogen receptors

TWO studies have been reported repudiating the long-held dogma that, in the absence of oestrogen, the oestrogen receptor is confined to the cytosol^{1,2}. The new model¹⁻³ supplants this interpretation with one in which unfilled receptor occurs exclusively in the nuclear compartment. Unfortunately, the new model may be just as full of artefacts as the old (see refs 4, 5 and below).

First, the new model, admittedly '... based on limited data and ... therefore rather speculative ...', fails to take account of approximately 100 publications demonstrating, by methods including analytical subcellular fractionation, affinity binding to intact cells and photo-affinity procedures, intrinsic localization of receptors for steroid hormones in extranuclear membranes of target cells, including the plasmalemma (from which the macromolecules are extracted by the conventional methods of preparing cytosol). This body of evidence has been reviewed extensively⁶⁻⁹. The Gorski group dispenses with these (uncited) potentially contradictory observations by the unsupported assertion that neither their laboratory² nor that of King and Greene¹ see "... evidence of binding [sic] of receptors to plasma membranes, a site that has been suggested in the past"³.

Second, the immunocytochemical distribution of 70-95% of oestrogen receptor with monoclonal antibody in (or on?)⁴ the nucleus, without cytoplasmic staining¹, in uteri of immature or pseudopregnant rabbits or of rabbits only 8 days after ovariectomy, and in a mammary tumour from a postmenopausal woman, is in direct contradiction⁴ to reports from the same laboratory within the past 18 months¹⁰. Since the fixation methods were essentially equivalent (L. Zamboni, personal communication), this variable seems excluded as the basis for the complete turnaround. The remaining differences between the successive observations were the use in the current work¹ of frozen sections, notorious for introduction of membrane distortion, and a different monoclonal antibody. It is difficult to assess the relative contribution of these additional variables. Alternative fixation-processing methods may well be required for good preservation of cell ultrastructure in the extranuclear regions without sacrifice of immunoreactivity.

Third, in the case of the report from the Gorski laboratory, there are abundant indications that the nuclear localization of 'unfilled' receptor was associated with profound perturbations of the native state of the GH₃ tumour cells used as oestrogen targets. Thus, the time-consuming enucleation process¹¹ on which the conclusions rest was carried out on Percoll-density-selected cells that were then preincubated at 37 °C for 45 min in the presence

of purportedly subtoxic concentrations of cytochalasin B and dimethyl sulphoxide, followed by 45 min centrifugation at 37 °C in a shearing gradient of Percoll and additives¹¹. The antibiotic¹², its solvent¹³, the Percoll itself^{14,15}, as well as the elevated temperature⁷, required to separate the major cellular compartments, are each known to perturb the cell surface. The increased endocytotic activity associated with such nonspecific stimuli, especially in tumour cells exhibiting an unusually high degree of basal pinocytosis, is well documented⁷. Nuclear translocation of resultant vesicles or macromolecular complexes by specified routes⁷ would be expected to promote concentration of receptor from plasmalemmal and other extranuclear sources.

Fourth, in the above work, the relative integrity of the nucleoplast fraction² was, surprisingly, not established by analyses for enzyme markers characteristic of other cellular compartments. Indeed, it is generally recognized that extranuclear membranous material is closely associated with the outer nuclear envelope on separation of the latter organelle by several procedures, even surviving shearing through heavy sucrose. Thus, removal of the outer nuclear membrane is required for definitive studies of nuclear composition (ref. 16 and citations therein). By inspection of the stained preparation², as well as by definition³, the nucleoplast, consisting of '... nuclei plus a small amount of cytoplasm plus intact membrane ...' is an incompletely characterized fraction.

Fifth, although, at first glance, the analogy drawn by the recent papers¹⁻³ to 'unfilled' receptors for thyroid hormone being concentrated in the nuclear fraction of its target cells seems compelling, neither group takes note of the equally conclusive data on the initial binding of triiodothyronine to the cell surface, followed by clustering and internalization, apparently in vesicular form^{9,17}. Similar observations are available for specific surface binding of other relatively hydrophobic agonists, including ouabain¹⁸ and oestradiol-17 β (refs 6, 19). These findings bear a close analogy to the emerging data for peptidic effectors⁷. Indeed, if primary recognition of blood-borne oestrogen were to be effected only within the confines of the nucleus, one is left to ponder the nature of the long-range forces that could underlie such a remarkable sensing and migration mechanism in 'target' cells.

Finally, we welcome as long overdue⁶ critical re-evaluation of the unmodified, traditional model of cytosolic localization of unbound receptor for steroid hormones, useful as it has been. However, it would seem prudent not to throw out the baby with the bathwater.

So McClellan *et al.*²⁰ have failed to present data on localization of oestrogen receptors in ovariectomized monkeys. In a personal communication that we have her permission to cite, McClellan has

stated that 'there was cytoplasmic staining in spayed controls, but it was not well differentiated from background', and that, presumably for the above reason, their group had 'postponed work on ovariectomized monkeys for more detailed study'. As is widely acknowledged, it is necessary to use great vigilance to combat the growing problem of contamination of animal quarters with even those low levels of oestrogen that promote nuclear association of transformed receptor.

Clearly, much remains to be done before the problem of subcellular distribution of oestrogen receptor in normal target cells in the absence of hormone can be firmly resolved.

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GREENE AND KING REPLY—Although an initial report¹ from this laboratory contained micrographs showing immunocytochemical localization of oestrogen receptor in the cytoplasm of tumour cells from paraffin-embedded breast cancers, we later found that this staining was not specific and could be mimicked by several unrelated polyclonal and monoclonal rat antibodies. In addition, essentially all cytoplasmic staining could be abolished by including suitable carrier proteins (for example, 10% normal goat serum) in the antibody solutions. Extensive work by us and others²⁻⁶ since then, with 10 unique monoclonal antibodies to human oestrogen receptor, indicates that specific staining for the receptor is confined to the nuclei of target cells in all conditions studied. These include at least 20 different

methods of fixation performed on frozen tissue sections, paraffin-embedded sections, vibratome thin sections and intact cultured tumour cells.

Szego's comment regarding the equivalence of paraffin-embedded materials to those discussed in our report is an oversimplification. The immunocytochemical literature contains ample evidence that many antigens easily localized in frozen or fresh tissues are obscured by the more extensive fixation and processing required for the preparation of paraffin blocks^{7,8}. We have already shown³ that extended fixation of oestrogen-receptor-containing tissues results in a progressive loss of nuclear staining for the receptor, with no change in the nonspecific extranuclear staining. With regard to the question of whether oestrogen receptors are located in or on the nucleus, recent electron micrographs (in which the ultrastructure is well preserved) show receptor localized in the chromatin and not on the nuclear (or other) membrane⁹. We cannot rule out the possibility that low levels of membrane-bound or diffuse cytoplasmic receptor have been missed by the immunoperoxidase method used, especially at the light microscopic level.

Finally, it is important to note that our findings are consistent with the hypothesis¹ that interaction of receptor with steroid induces the formation of a complex that binds more tightly to the nucleus and that this 'activated' steroid-receptor complex is responsible for the biological effects of the hormone.

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GORSKI REPLIES—In our original paper¹, we did not comment on membrane localization of steroid receptors, but in our review article² we pointed out that the cytoplasts contain intact cell membranes yet do not contain appreciable concentrations of oestrogen receptors. The cytoplasts prepared from GH₃ cells contain normal concentrations of cytosolic marker proteins, exclude dyes and can incorporate labelled amino acids into proteins and

specifically prolactin.

These protein synthetic activities require transport of, for example, amino acids and energy sources, which we assume requires a reasonably normal membrane, and the dye exclusion also suggests an intact membrane. Our observations plus those of King and Greene³ and more recently McClellan *et al.*⁴ provide no support for a membrane-localized receptor. I believe that there is no substantive support in the literature for membrane localization of steroid receptors.

Finally, the concern of Szego and Pietras about steroids getting into cells is surprising. They readily get out of the cells where they are synthesized and they readily pass through many layers of cells during tissue incubations. There is nothing in most normal cells that one would imagine to be an effective barrier to diffusion of steroids unless one conceptualizes a cell as being simply a lipid sack surrounding a drop of water.

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Mobility and evolutionary variability factors in protein antigenicity

IN a recent *Nature* article¹, a correlation was made between segmental mobility and the location of continuous antigenic determinants in proteins. Temperature factors obtained from X-ray crystallographic data were used as a measure of local conformational flexibility for three proteins, namely, the tobacco mosaic virus (TMV) protein, myoglobin and lysozyme. The antigenic sites had been deduced previously from a number of studies that examined the binding of peptides to anti-protein antibodies.

Can generalizations about the total antigenicity of a protein be made from studies that rely heavily on peptide binding to detect antigenic sites in proteins? In assessing the binding of antibodies to a protein, it seems reasonable that regions of local flexibility are more likely to be mimicked by linear peptides than are regions that are more rigid. A peptide adopts an ensemble of conformational states in solution, some of which may be close to the conformation of that sequence as it appears in the native antigen. Thus, some peptide molecules may be induced into a conformation that fits an antibody-combining site. Studies in which peptides

are used to identify antigenic sites, are, therefore, biased towards detecting epitopes that are flexible. As Westhof *et al.* pointed out, it is possible that the anti-protein antibodies that bind peptides do not represent all of the antibodies present in the antisera in the studies they cited¹.

Another method that is used extensively to study protein antigenicity involves comparison of evolutionarily related proteins such as lysozyme, myoglobin and cytochrome *c* in fine specificity studies². Generally, the results of this type of analysis have shown a correlation between antigenicity and evolutionary variability^{3,4}. The detection of antigenic sites by such fine specificity studies, however, may be biased towards detecting antigenicity in regions of evolutionary variability. Naturally occurring amino-acid substitutions within protein families are likely to occur in regions of polypeptide chain flexibility as natural selection clearly favours those mutations that do not either perturb the overall tertiary structure of a protein or reduce its functional capacity. Surfaces of close contact within the packed secondary structure in the interior of a protein are likely to be both inflexible and structurally conserved evolutionarily. In contrast, regions of the polypeptide chain in which changes in local conformation arising from point mutations can be tolerated are most likely to be on the surface, where they are not involved in the long-range interactions that stabilize the internal folding of the molecule, and, hence, are more likely to be flexible. It is probable that antigenicity, polypeptide chain flexibility and evolutionary variability are related. Therefore, correlations that have been made between atomic mobility in proteins and antigenic sites for anti-protein antibody responses^{1,5,6}, although significant, may be explained by factors other than an inherent ability of flexible regions to invoke an immune response.

A complication that arises in correlating published data on the antigenicity of proteins with their molecular mobility is that many protein antigens that have been studied have a high degree of homology with the host animal's protein. The TMV protein cited in ref. 1 is an exception. In general, antibodies elicited in a particular host appear to arise in response to regions of the immunogen that differ in amino-acid sequence from the homologous host protein²⁻⁴. This phenomenon derives from immunological tolerance⁷ and is particularly well documented for cytochrome *c*, but it also seems to hold for myoglobin, insulin, and other well-characterized protein antigens in mammalian hosts². Certain sites on a molecule may not elicit an immune response even though they are flexible, if B cells reactive to these regions are not present as a result of either tolerance⁷ or possible evolutionary effects on the germline repertoire⁸.

Existing methodologies for studying antigenic sites cannot completely deter-

mine all of them or all of the residues within an individual antigenic site on a protein that are involved in binding an anti-protein antibody. Ultimate resolution will rely on more refined methods such as X-ray crystallographic studies of antigen-antibody complexes, as reported recently⁹. Further data are required to determine whether the correlation observed between mobility and antigenic sites in proteins^{1,5,6}, while significant, is also of immunological importance.

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VAN REGENMORTEL AND ALTSCHUH
REPLY—Jemmerson and Paterson imply that Westhof *et al.*¹ have attempted to link the total antigenicity of proteins with regions of local mobility, and they suggest that such an attempt is bound to fail because anti-protein antibodies that bind peptides may not represent all of the antibodies present in the antisera. We wish to point out that there is no mention of total antigenicity in our paper and that, on the contrary, we stated clearly that "segmental flexibility in proteins emphasizes only one type of antigenic determinant", namely the type of continuous epitope that can be mimicked with short peptides. We also suggested that continuous epitopes are probably less numerous than the discontinuous epitopes revealed in the studies with monoclonal antibodies.

We agree that our study using short peptides was biased towards the detection of flexible epitopes. However, any experimental study of protein antigenicity will necessarily suffer from one operational bias or another. The monoclonal antibodies used in X-ray crystallographic studies of antigen-antibody complexes are selected for high binding affinity and are not representative of all the antibodies produced during the immune response; they will, therefore, present a biased view compared with the interactions that occur with low-affinity antibodies. A study of the antigenicity of related mammalian proteins that are subject to immunological tolerance can also be said to be biased compared with the study of antigens such as plant viruses, which are free from such restrictions. Each experimental approach used in the study of protein antigenicity

will emphasize a particular aspect of the many types of interaction that constitute immunological recognition.

In view of our ignorance regarding the total antibody repertoires relevant for different protein antigens, it seems to us pointless to argue about the relative importance of these different experimental approaches. However, present interest in mimicking protein epitopes by means of short synthetic peptides underlines the immunological relevance of any observations that link mobility to the location of continuous epitopes in proteins.

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TAINER ET AL. REPLY—Jemmerson and Paterson suggest a correlation between evolutionary variability and antigenicity, while noting that such variable regions are likely to be subject to fewer structural constraints. Their point is well taken for the generalization of the relationship between local mobility and antigenicity of protein sites, as identified by Westhof *et al.* for TMV¹ and ourselves for myohaemerythrin², to other proteins that are affected by tolerance. The influence of tolerance is undoubtedly crucial in the majority of protein systems studied by immunologists. Currently, there appear to be a number of related structural parameters that bias antigenicity including sequence variability, hydrophilicity, solvent-accessibility, shape, and local mobility^{3,4}, but it is difficult to determine which is the primary underlying correlate. The protein myohaemerythrin is a good model for antigenicity studies because it is not subject to tolerance, and it is unique in having highly accurate temperature factors corrected for the effects of crystal contacts⁵. We designed an experiment to address the specific question of why anti-peptide antibodies can recognize the native protein, and found a better relationship for local mobility than for these other parameters.

For anti-peptide antibodies, the parameters of hydrophilicity, shape and variability cannot explain what we term the order/disorder paradox, that is, how can an antibody raised against a presumably disordered peptide recognize the native, highly ordered protein? The results of our experiment help to resolve this paradox by suggesting that the antigenic sites of proteins should not be viewed as static, fixed conformations. Evidence from NMR and hydrogen/deuterium exchange rates also blurs the order/disorder distinction by revealing the differential mobility of protein sites³. The role of flexibility^{1,2,6} in facilitating the interaction of antigen with antibody adds the dimension of time to

understanding the thermodynamics of antigen-antibody union. The correlation of flexibility with sequence variability, hydrophilicity, solvent-accessibility, and shape could account for the correlation of these related parameters with antigenicity, while simultaneously explaining the success of anti-peptide antibodies in recognizing the parent protein. Finally, the knowledge that mobility is an intrinsic molecular parameter affecting antigen-antibody union has implications for any model of antigenic recognition in that it implies a multiple-step process rather than a single collision resulting in simple lock-and-key complementarity.

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Psychology

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Applied Biological Sciences

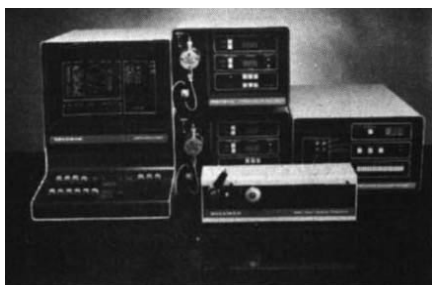
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What's new in the chemistry lab?

The American Chemical Society is meeting in Chicago on September 8 to 13. Here are some new products of interest to chemists.

- The Beckman Instruments, Inc. System AA-4, one of four new Beckman HPLC systems for amino acid analysis, is optimized for high-sensitivity, low-picomole microgradient analysis. System AA-4 includes two Model 114M pumps, the Model 421A controller, the Model 163 continuous variable wavelength detector and 2-mm internal diameter Ultrasphere ODS microbore column. System AA-4 provides consistent gradients delivered by high-precision Model 114M pumps, each capable of flow rates ranging from 1 μ l per mm to 30 ml per min. The system is optimized for 2mm microgradient HPLC with a μ -flow organizer that includes a zero dead volume injector and a low volume mixer. The Model 163 continuous variable wavelength UV detector and micro-flow cell offer excellent sensitivity. Storage and linkage of methods are simple with the Model 421A controller. The 2-mm ID Ultrasphere ODS microbore reversed-phase column provides high-resolution, high-sensitivity HPLC.

Reader Service No. 100.



Beckman's AA-4 is optimized to give high sensitivity low-picomole microgradient analysis.

- The Hilger Analytical Chromaspek M amino acid analyser provides the benefits of modern microprocessor technology and the sophistication of integration together in one system. Using gradient elution, the instrument requires only two easy-to-prepare buffers and up to ten user-defined pH gradient profiles are available for increased flexibility and speed in method development. Overall sample throughput is increased with the ability to analyse consecutive samples using any of the ten pH profiles. Fully annotated chromatograms are produced with corresponding final report printouts and results storage, providing greater flexibility and time saving in routine operation. Optional accessories

These notes are based on information provided by the manufacturers. To obtain further details about these products use the reader service card bound inside the journal.

available include high pressure loading, a refrigerated 60-position autosampler, fluorescent detection and a wide range of spares and consumable kits.

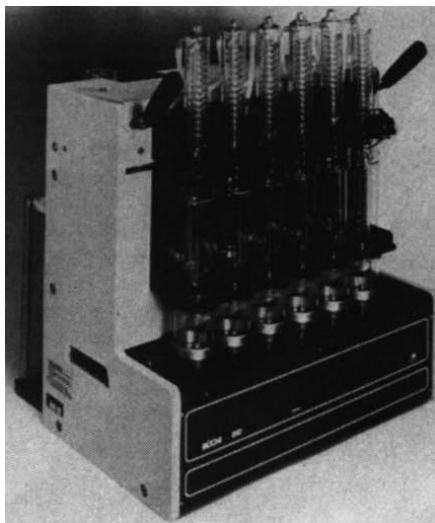
Reader Service No. 101.

- ICN Radiochemicals' multi-use liquid scintillation cocktail for aqueous samples is compatible with large volumes of water and is stable both in acid and alkali. Universol Cocktail has high efficiency and controlled chemiluminescence. It is a safe, non-flammable solvent system. Samples and technical information are available upon request.

Reader Service No. 102.

- The Brinkmann Instruments division of Sybron Corporation has now introduced the Büchi Soxhlet fat extractor to the US market. For laboratory procedures that include the determination of fat and oil content, the fat extractor uses a true Soxhlet method to improve results, simplify handling, decrease total extraction time and make fat extraction safer and easier. The fat extractor uses steam from a built-in or externally mounted generator as its source of heat, eliminating the dangers associated with other methods. For higher temperatures, the heating block can be heated with a commercially available circulator. Extractions can be performed on six samples simultaneously in two different sized extraction tubes holding up to 20 or 80 g of sample. Shorter extraction times are complemented by easier, more efficient solvent recovery. The recovered solvent is ready for use in subsequent extractions. For low-fat-content samples, Büchi produces a hydrolysing unit for use prior to extraction.

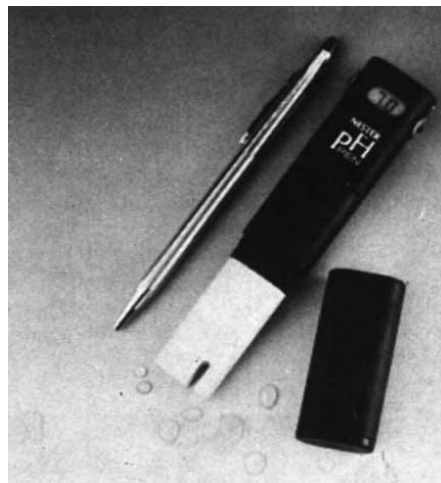
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The Büchi Soxhlet fat extractor.

- Nester Instruments has announced the availability of the pH PEN, a miniaturized pH meter that fits in a shirt pocket. The self contained meter utilizes a gel-filled combination glass electrode. The pH PEN displays measurements with large liquid crystal display characters that are visible even in direct sunlight. The unit weighs four ounces and is battery powered. It features advanced solid-state circuitry and a reading lock button to hold measurement values for recording.

Reader Service No. 104.



Nester Instruments' pH pen fits in a shirt pocket.

- Whatman diphenyl reversed-phase thin-layer chromatography (RPTLC) plates consist of juxta aromatic rings bonded through the silane to a special optimized pore silica gel. The mechanism of separation is reversed-phase partition based on hydrogen bonding from the aromatic rings, so these plates are extremely effective in separations of compounds with molecules having an aromatic character: steroids, cholesterol, PAHs, catecholamines. Diphenyl plates also provide excellent separations of biological molecules such as proteins and peptides and other biocompounds with molecular weights to 100,000 in simple isopropanol buffer systems. The plates are moderately fast with a durable surface. They are much less load-dependent than other RPTLC plates and can be used in both analytical and preparative TLC. No special techniques or procedures are required to use diphenyl plates. Spotting is simpler since the layer is much less hydrophobic than a C18 layer, development and visualization can be accomplished using lipophilic reagents. All RPTLC solvent systems are applicable and the water-isopropanol system widely used for proteins and other separa-

tions is appropriate for these diphenyl plates. This mechanism is an effective high-performance alternative to C18, ion exchange, or steric exclusion media for biological separations within the 100,000 MW range.

Reader Service No. 105.

• The new fully automatic BI-90 sub-micrometre particle sizer from Brookhaven Instruments Corporation gives size distribution of particles, macromolecules, and microemulsions, from 0.005 to 5 μm . Using laser light scattering, it incorporates an automatic signal level-controller, video monitor and electronic dust filter for accurate measurements of particle sizes, molecular weights and diffusion coefficients. The BI-90 is compatible with a full range of hardware and software.

Reader Service No. 106.



Brookhaven Instruments' BI-90 automatic sub-micrometre particle sizer.

• The Genzyme Corporation has introduced three new highly purified products for glycoprotein research — *O*-glycanase, endoglycosidase H and 1-deoxynojirimycin. The *O*-glycanase is purified from cultures of *Diplococcus pneumoniae*. It hydrolyses the gal β (1-3)galNAc core disaccharide linked serine or threonine in mucin-type glycoproteins. Both glycopeptides and glycoproteins are substrates for this enzyme. *O*-glycanase is available as a 25% glycerol solution in 15mM Tris-maleate buffer. Endoglycosidase-H is an enzyme essentially free of protease and exoglycosidase activity. It catalyses the hydrolysis of chitobiose core of high mannose and certain hybrid oligosaccharides. This product is available as a lyophilized powder. 1-Deoxynojirimycin inhibits the action of glycosidase I and II during the normal processing of N-linked glycoprotein. Cells incubated in the presence of 1 mM of deoxynojirimycin synthesize greatly reduced amounts of complex carbohydrate chains and increased amounts of high mannose chains. This inhibitor is available as crystalline salt.

Reader Service No. 107.

• Bio-Rad has introduced two new kits for analysing biogenic amines based on high-performance liquid chromatography — one for catecholamines, the other for metanephrines. The tests utilize a new HPLC technique in which a specially selected cation-exchange column efficiently separates the biogenic amines. In

the catecholamine test the Bio-Rex 70 extraction of free catecholamines from urine has been modified for HPLC analysis. The metanephrine test employs a new technique for sample preparation based on the formation of a stable anion by metanephrines in basic solution. Both methods use electrochemical detection because neither catecholamines nor methanephrines have strong ultraviolet absorbance or native fluorescence. In the catecholamine test, concentrations from 2 to 400 $\mu\text{g l}^{-1}$ can be measured; the concentration range in the metanephrine test is 10 to 2,000 $\mu\text{g l}^{-1}$. In both cases, the reproducibility of the test is generally improved by using an internal standard.

Reader Service No. 108.

• Omega offers three new flow metering, monitoring and control systems. The FV4000 flowmeter, FC5000 flow computer and DP60 flow rate indicator/totalizer can be used alone or as an integrated system. The FV4000 vortex flowmeter measures liquids, gases and steam with $\pm 1\%$ accuracy of reading. It features a piezoelectric sensor and a choice of 4–20 mA linear output, scaled or unscaled pulsed outputs. When connected to the FC5000 flow computer, it provides continuous on-line compensated true mass flow rate. Housed in a NEMA 4X case with a clear plastic cover, the FC5000 features two field-selectable standard compensation calculations and can be programmed to meet specific application requirements as well. Totalized flow is displayed in either pounds for steam, or standard cubic feet for gases. The flow rate is monitored on a second indicator from 0 to 100%. For liquid flow applications, FV4200 Series is supplied with a 4–20 mA output for use with the DP60 Series digital panel meters. These come in two models for totalizing and batch controlling or use as a rate indicator with a relay alarm.

Reader Service No. 109.



Three new flow control systems from Omega.

• Sartorius Filters Inc.'s Miniultrasart is a self-contained ultrafiltration module with a 20,000 M^w cut off for depyrogenation of aqueous solutions and the concentration of proteinaceous fluids. It is supplied sterile and pyrogen free, and complies with USP and CGMP requirements. The module contains one square foot of hydrophilic, asymmetric cellulose triacetate membrane. The unit is designed to utilize a

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• Lab Safety Supply is offering a free sample of their new 'Vapour Barrier Sorbent' (VBS) blankets that absorb hazardous liquids and suppress vapours at the same time. These sheets of treated microfibres of polypropylene have a high absorbency and exceptional chemical resistance. A polyethylene film on one side helps suppress toxic, irritant or flammable vapours. VBS blankets can absorb spills of acids, caustics, solvents, oils and even hydrofluoric acid. They can also be used to protect work surfaces from chemical spills.

Reader Service No. 111.



A VBS blanket in action.

• A new brochure entitled "Megabore the Packed Column Alternative" is now available from J & W Scientific Inc. The 32-page brochure gives details on the company's Megabore column for gas chromatography. Technical information includes chromatograms that compare results with those obtained using traditional packed columns. The Megabore column comes in two convenient lengths for optimal speed and resolution, with a choice of seven different bonded stationary phases. A selection guide gives all the details needed for selecting Megabore columns. Useful data are provided on applications. Accessories for gas chromatography are also described.

Reader Service No. 112.

• Packard Instrument is giving away its latest application report describing the metabolic profiling of urinary organic acids and steroid hormone metabolites. All experiments were performed using a fused silica microbore column, measuring 10 m × 200 µm mounted in a standard Packard Model 436 gas chromatograph. Interest in microbore columns is increasing since, compared to standard capillary columns, they offer improved resolution or shorter analysis times. But they require excellent oven-temperature stability, minute over-temperature gradients and ultra-fast response from the instrument's electronics. Packard gas chromatographs meet these stringent requirements and accept microbore columns without mod-

ification, the company says. The report describes the experimental conditions and is illustrated with six chromatograms. Three profile urinary organic acid derivatives with characteristic pathological patterns and the others illustrate one normal and two pathological profiles of steroid hormone metabolites.

Reader Service No. 113.

• Perkin-Elmer have expanded their TADS Series thermal analysis range with the introduction of a new DSC-4 robotic system. It is compatible with new and existing DSC-4 differential scanning calorimeters. Using this robotic system, it is now possible to run 48 DSC samples in any order, without operator intervention. The system is composed of a removable, 48-position sample carousel and a pneumatically controlled robotic sampling arm.

Reader Service No. 114.

• Universal Scientific is now producing reversed-phase thin-layer chromatography (RPTLC) plates. The great advantages afforded by the use of reversed phases in high performance liquid chromatography are now leading to the adoption of this technique in thin-layer chromatography too. Universal's plates are made with a mineral binder capable of unlimited use in all potential applications of mobile phases rich in water, up to 100%, or buffer solution, ion-pair chromatography, and with detectors of any type.

Reader Service No. 115.

• The new HP 3393A computing integrator from Hewlett-Packard adds advanced software to the HP 3390 integrator series and provides extensive data-handling capabilities for the analytical laboratory. It can be used as a companion to gas or liquid chromatographs or other analytical instruments where accurate integration and advanced decision-making capabilities are important. The new system not only integrates, calculates and reports, it also re-integrates, replots and performs multi-level calibration. The programming language is BASIC. An alphanumeric keyboard, thermal printer/plotter and RS-232-C, INET (instrument network) and sample number-input communication/control interfaces are built-in.

Reader Service No. 116.



Hewlett-Packard's HP 3393A.

• Hamamatsu's new streak tube, for use with the universal streak camera, extends streak experiments to 1.5 µm. Three plug-ins include: a synschronscan plug-in with a

high speed repeatable sine-wave drive deflection; a fast plug-in, with time resolution of better than 2ps for ultrafast kinetics experiments; and a slow plug-in, providing a larger time window for recording phenomena out to 1ms full scale. This new streak tube is a high sensitivity device with built-in microchannel plate for multiplication of photoelectrons and weak signal detection. No external image intensifier is needed. The C1587-01 can be used with a camera back for photographic recording of the output or with a number of temporal analyser electronic readout systems.

Reader Service No. 117.



Hamamatsu's C1587-01 streak camera.

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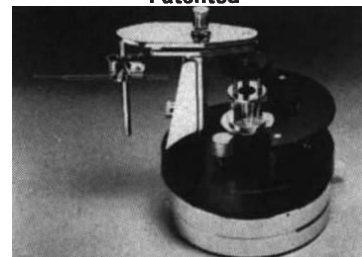
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Reader Service No.22

Graduating in Australia

from Richard Pearson

A study just published in Australia gives a useful pointer to the status of graduates in the employment market.

HIGHER education throughout the Western world is being encouraged to increase its relevance to and links with industry. One of the 'performance factors' being used to measure the effectiveness and relevance of higher education is the employment rate for newly qualifying graduates. Many countries now collect some basic data on the initial employment or other destinations a few months after graduation, but in very few has there been any serious assessment of the longer-term employment pattern of graduates. While the initial destination statistics are very valuable labour market indicators, they take no account of the fact that the initial transition into employment can be an extended process. Not all graduates seek jobs straight away, others take a temporary job while awaiting the 'right' opportunity, while yet others quickly change jobs and careers in the year or two after graduation.

A recent study of Australian graduates five years after qualification is therefore to be welcomed for the insights it shows into the working of the labour market (P. Coyte, *Graduates in the Labour Market*, University of Sydney, 1985). The study, based on those graduating in 1979 from five Australian universities, gives details

Table 1 Unemployment after graduation

1975	9.5%
1977	13.4%
1979	15.2%
1981	13.4%
1982	14.3%
1983	20.1%
1984	15.8%

Figures from *Graduates in the Labour Market* (University of Sydney, 1985).

of over 4,000 graduates and their careers. In particular the study looked at the type of employment obtained, relative pay, unemployment and mobility.

In common with their contemporaries in many other countries, Australian graduates have been suffering a deterioration in their job prospects on graduation over the past few years. As Table 1 shows, their unemployment rate on graduation rose to one in five in the period up to 1983 but since then has improved somewhat. On the salary front, graduates' starting salaries have declined by about 20 per cent over the past six years relative to average earnings (Fig. 1).

However, in terms of longer-term prospects, the study shows that unemployment

is not an issue. Only 3.2 per cent of those graduating in 1979 were unemployed in 1984 compared with the national rate of 10 per cent and the 9 per cent who were still seeking work six months after graduation in 1979. At the time of graduation, subjects with above average unemployment rates included psychology, biological, agricultural and earth scientists and engineers, all these groups suffering unemployment rates in excess of 13 per cent. By 1984 the worst unemployment rate was

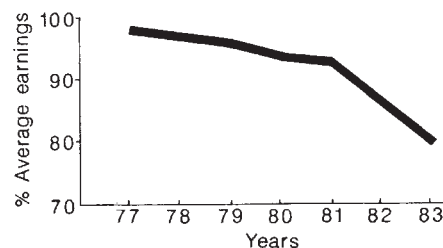


Fig. 1 Salaries on graduation in relation to average weekly earnings.

that being experienced by the earth scientists, but only at 6 per cent, all the other high unemployment subjects having improved to around 3-4 per cent. Engineers had improved their position even more and fewer than 2 per cent were unemployed in 1984.

In employment terms, the dominant sector has been government itself, which took 30 per cent of all those graduating and more than half of those going into employment. By 1984, five years later, nearly half of the graduates were in government employment with the number employed in education having doubled from some 10 per cent in 1979 to over 20 per cent in 1984. Industry and commerce's share of the graduates was rather smaller in both years, 11 per cent rising to 16 per cent. The increasing percentages in employment between the years are accounted for by those choosing postgraduate studies entering the labour market in subsequent years.

If graduates are advantaged in overall employment terms then the survey shows that their salaries are also higher than for the population at large. While most graduates were earning 10-20 per cent above average weekly earnings five years after graduation, typically earning \$23-27,000 annually, it was notable that the doctors and dentists were, at over \$33,000, receiving more than 50 per cent over the average (Table 2). However, apart from these exceptions it is remarkable how little distinction was found between the relative

earnings of graduates in different subjects. This suggests there may be significant institutional rigidities in graduate salaries in the labour market, possibly because of the dominant nature of the public sector employers, in particular education, and their standard pay scales. These figures suggest that an assessment

Table 2 Annual salary five years after graduation (1984)

	\$A
Medicine	35,000
Dentistry	33,050
Law	27,400
Engineering	26,501
Computer science	26,250
Psychology	24,500
Physical science	24,000
Bio science	23,002
Pharmacy	21,017
All graduates	25,690
Average annual earnings	21,200

of relative earnings will not help prospective students choose between subjects. One anomaly is that pharmacists, although they have consistently had the lowest unemployment rates and easiest transition into employment, have the lowest salaries. This may be 'cause and effect' — low salaries meaning increased job opportunities — but at any rate reliance should not be placed on a single labour market indicator when assessing labour market prospects.

A significant majority — 59 per cent — of graduates had changed jobs in the five years since 1979, with the government sector having the highest retention rate. Once these graduates did decide to change jobs they were likely to leave the government service altogether, in contrast to those leaving industry and commerce who were more likely to have moved within that sector. The report found no dominant cause of mobility, with personal factors such as travel or further study being frequently mentioned, but salary seemed not to be an issue.

The report concludes that graduates are still advantaged in the labour market, whether due to the educational process or their inherent abilities is not clear, but that such advantages have deteriorated in recent years. Apart from dentistry and medicine, the distinctions between subjects appear less marked (see *Nature* 310, 260; 1984) than would be suggested by research from other countries. □

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